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Original Article

PHYTOCHEMICAL STUDIES OF DIFFERENT PHASES OF GERMINATION OF *NIGELLA SATIVA* LINN - A MEDICINALLY IMPORTANT PLANT

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ABSTRACT

Objective: Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. The present paper deals with the phytochemical screening of medicinally important compounds from *Nigella sativa*, an important medicinal plant, in its different germination phases. This study involves the preliminary screening, quantitative determination and the qualitative thin layer chromatographic separation of some important secondary metabolites from the seeds (collected from market) and its different germinating stages till the complete seedling with two leaves arose.

Method: A qualitative and quantitative phytochemical analysis was performed for the detection of various bioactive compounds viz. alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins through standard biochemical assays and thin layer chromatography.

Results: Germination dramatically decreased alkaloids, sterols, terpenoids and cardiac glycosides content while amount of phenols, flavonoids and saponins significantly increased in sprouts in a time-dependent manner. The quantitative analysis showed that the concentration of phenols (23.75mg/ g FW), and flavonoids (24.90 mg/g FW) reached the peak on 5th and 6th day of germination respectively, almost 3 times higher than the initial concentration in seeds ($p < 0.05$).

Conclusion: Therefore, the germination of *Nigella sativa* seeds significantly increased some of the important phytochemical contents and decreased antinutritional components. The generated data has provided the basis for its wide use as the therapeutic agent both in the traditional and folk medicines.

Keywords: Thin layer chromatography, Secondary metabolites, Alkaloids, Flavonoids.

INTRODUCTION

Plants and plant-based compounds are the basis of many of the modern pharmaceuticals used today for the treatment of various dreadful diseases. The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self experiment. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant [1]. According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [2].

Nigella sativa L. is commonly known as black seed belongs to the botanical family of Ranunculacea. Its seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world for acquiring good health and treating many ailments including fever, common cold, headache, asthma, rheumatic diseases and various microbial infections and to expel worms from the intestine [3, 4, 5]. *N. sativa* is considered a natural food additive and a condiment. It is typically consumed mixed with honey and in baking products or pastries. Also, it had been used for medicinal purposes as a natural remedy in many ancient cultures. It is included in the list of the natural drugs of Al-Tibb AL-Nabawi as it was recommended by the prophet Mohammed (PBUH), "The *N. sativa* is the medicine for every disease except death." [6, 7]. It was also reported that the pharmacologically active constituents of the *N. sativa* are mainly nigellone, thymoquinone, dithymoquinone, thymohydroquinone, and thymol [6]. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies [8]. Many plant constituents are known, many other that remains undiscovered, especially in germinating seeds are beyond the imagination. So, the present study was carried out to investigate the phytochemicals present in successive germinating phases of *N. sativa* seeds.

MATERIALS AND METHODS

Collection of *N. sativa* seeds

Seeds of *N. sativa* were procured from a local grocery store in Lucknow and were certified by the National Botanical Research Institute, Lucknow, India.

Germination of *N. sativa* seeds

Seeds of *N. sativa* were grown in glass petriplates having two or three folds of damp blotting paper in distilled water, at room temperature of about 28±2˚C under control conditions. 0.25gm seeds were inoculated in each petriplates under aseptic conditions. The seeds were incubated in dark till sprouting was initiated (3 days) after which the plates were transferred to culture room at a light intensity of 100 µmol $m^{-2}s^{-1}$and a 14/10 h (day/night) photoperiod till the development of plantlet with two leaves.

Germination, defined as 1 mm radicle emergence, was followed for 11d; no contamination by microorganisms was observed during this time.

Preparation of samples of each germination stage

The seeds were kept for germination and the sample was collected each day till complete germination took place. The samples were shade-dried and ground to a fine powder which was stored in air-tight plastic bags. The powder was used for phytochemical tests, spectrophotometric analysis and thin layer chromatography.

Phytochemical Investigation

The phytochemical properties were determined by the following methods. [9, 10, 11, 12]

1. Test for Sterols

a. Salkowaski reaction

0.5mg of each residue of each extract was taken in 2ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test-tube. The test-tube was shaken for few minutes. The development of red color in the chloroform layer indicated the presence of sterols.

2. Tests for Alkaloids

a. Hager's reagent

A saturated aqueous solution of picric acid was used for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained, indicating the presence of alkaloids.

b. Wagner's reagent (Iodine-potassium iodine)

1.27gm of iodine and 2gm of potassium iodide were dissolved in 5ml of water and the solution was diluted to 100ml with water. When few drops of this reagent were added to the test filtrate, a brown flocculent precipitate was formed indicating the presence of alkaloids.

3. Tests for Tannins

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents.

a. **Ferric chloride reagent**

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Dark green color was obtained showed the presence of tannins.

b. **Lead acetate test**

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitation was obtained showed the presence of tannins.

4. Tests for Saponins

a. Foam test

0.5 mg of test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. The formation of froth indicated the presence of saponins.

5.Tests for Phenols

a. **Ferric Chloride test**

1 ml extract was taken in water and warmed then 2 ml of ferric Chloride (FeCl3) were added. The formation of green and blue colour solution indicates the presence of phenolic compounds.

b. **Lead Acetate test**

Extract was added with 2 ml of lead acetate. The formation precipitate indicates the presence of phenolic compounds.

6. Tests for Flavonoids

two methods were used to determine the presence of flavonoids in the plant sample.

a. Few drops of 1% ammonia solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids.

b. A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

7. Test for Terpenoids

5ml of each extract was mixed in 2ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

8. Test for cardiac glycosides

5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Study of Phytochemicals by Thin Layer Chromatography [13, 14]

I. TLC study of sterols

Two grams of powdered samples of different days of germination were extracted with 10ml methanol in water bath (80°C/15 min).The condensed filtrate is used for chromatography. The sterols were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour and Rf values of these spots were recorded under visible light after spraying the plates with anisaldehyde-sulphuric acid reagent and heating (100˚C/6 min).

II. TLC study of alkaloids

The powdered samples of different days of germination were wetted with a half diluted NH_4OH and lixiviated with EtOAc for 24h at RT. The organic phase is separated from the acidified filtrate and basified with NH_4OH (pH 11-12). It is extracted with chloroform (3X), condensed by evaporation and used for chromatography. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1). The colour and Rf values of the separated alkaloids were recorded both under ultraviolet (UV254nm) and visible light after spraying with Dragendorff's reagent.

III. TLC study of phenols

The powdered samples of different days of germination were lixiviated in methanol on rotary shaker (180 thaws/ min) for 24h. The condensed filtrate was used for chromatography. The phenols were separated using chloroform and methanol (27:0.3) solvent mixture. The colour and Rf values of these phenols were recorded under visible light after spraying the plates with Folin-Ciocalteu's reagent heating at 80°C/10min.

IV. TLC study of flavoniods

One gram powdered samples of different days of germination were extracted with 10ml methanol on water bath (60°C/ 5min). The filtrate was condensed by evaporation, added a mixture of water and EtOAc (10:1 ml), and mixed thoroughly. The EtOAc phase thus retained was used for chromatography. The flavonoid spots were separated using chloroform and methanol (19:1) solvent mixture. The colour and Rf values of these spots were recorded under ultraviolet (UV254nm) light.

V. TLC study of glycosides

The powdered samples of different days of germination were extracted with 70% EtOH on rotary shaker (180 thaws/min) for 10h. 70% lead acetate was added to the filtrate and centrifuged at 5000rpm/10 min. The supernatant was further centrifuged by adding 6.3% Na_2CO_3 at 10000 rpm/10min. The retained supernatant

is dried, redissolved in chloroform and used for chromatography. The glycosides were separated using EtOAc-MeOH-H2O (80:10:10) solvent mixture. The colour and Rf values of these spots were recorded by observing under ultraviolet (UV254nm) light.

SPECTROPHOTOMETRIC ANALYSIS

Preparation of fat free sample

1g of the samples of each germination stage was defatted with 50 ml of diethyl ether using a soxhlet apparatus for 2 h.

Estimation of total phenols in different stages of germination

Total phenols were determined by Folin Ciocalteu reagent [15]. A dilute extract of each plant extract (0.5 ml of 1:10 g/ml) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/l solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of DW), which is a common reference compound.

Estimation of total flavonoids in different stages of germination

Aluminum chloride method was used for flavonoids determination [16, 17]. Each plant extract (0.5 ml of 1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100g /l in methanol.

RESULTS

Germination of Seeds

In morphological study of seed germination, it was seen that sprouting started from 4th day of imbibition and on 11th day, complete plantlets with two leaves were developed. Germination rate was approximately 95 %. The phytochemical analysis carried out on the dry sample of different germination phases of *N. sativa* seed extracts showed the significant change in some bioactive compounds. In eleven extracts of samples of germination phases, eight bioactive constituents were tested. The results of effect of seed germination on alkaloid composition of *N. sativa* seeds are presented in the Table 1.

Qualitative analysis showed that the germination caused a clear decrease of total alkaloid contents as the germination proceeds. The sterol content was high in seeds and early phases of germination which was followed by a decrease as germination proceeds. Polyphenols were found to be accumulated during the commencement of imbibition. The compound was found to be present in good quantity in the samples of 4th and 5th day of germination after that it decline continuously. Phytochemical analysis showed the presence of phenols and flavonoids in all the samples, these compounds were increased in early phases of germnation, reaching maximum on 5th-6th day followed by a continuous decrease. Analysis of tannins in the later stages of germination was found to be positive but higher colour intensity was observed in the extracts of 8th, 9th, 10th and 11th day. Analysis of saponins and cardiac glycosides showed its presence in the initial stages of seed germination, while it was not observed from 7th day of seed germination; persistent frothing was intense in the seed extract and the extracts of 1st to 4th day of germination, it indicated the presence of saponins in good quantity in the samples of early germination phases.

Table 1: Preliminary screening of secondary metabolites from *N. sativa*

Day	Alkaloids	Sterols	Phenols	Flavonoids	Tannins	Saponins	Terpenoids	Cardiac Glycosides
0	+++	+++	+	+	-	++	+	++
1	++	+++	++	+	-	++	+	++
2	++	++	++	+	-	++	+	++
3	++	+++	++	+	-	+++	+	+
4	++	++	+++	++	-	+++	+	+
5	++	++	+++	+++	+	+++	+	+
6	++	++	++	+++	+	++	+	+
7	++	++	++	++	+	+	+	+
8	+	+	++	++	++	-	+	-
9	+	+	++	+	++	-	-	-
10	-	+	++	+	++	-	-	-
11	-	+	++	-	++	-	-	-

* '-' symbolizes absence of the metabolite; *'+' symbolizes presence of the metabolite; *'++' symbolizes moderate presence; *+++ symbolizes good presence.

Thin layer chromatography of different phases of germination of *N. sativa* seeds

Biochemical analysis of phytochemicals during different germination stages of *N. sativa* showed the significant presence of alkaloids, sterols, phenols, flavonoids and cardiac glycosides which were further analyzed by thin layer chromatography. The data of qualitative separation of alkaloids of *N. sativa* seeds during germination by thin layer chromatography is tabulated in Table 2. Seven alkaloids were seen as distinct bands on the TLC plate as depicted in Table 2. Out of these three bands (Rf=0.588, 0.765, 0.882) were visible in the visible range of light whereas four fluorescent alkaloids (Rf =0.067, 0.588, 0.765, 0.882) were reported in various germinating stages of the seed. There were two spots of alkaloids during first two days of germination which increased to four from 4th day till 8th day which further increased to six on 9th day after which the alkaloid content decreased till the formation of seedling. But, two new bands (Rf = 0.647 & 0.676) were seen from 9th day of germination. This may be perhaps due to the inter conversion of these compounds into other derivatives.

The data of qualitative analysis of sterols of *N. sativa* seeds during germination by thin layer chromatography is tabulated in Table 3. It has revealed the presence of four sterols in all the samples (Rf =0.063, 0.344, 0.750 & 0.875) while only three spots of sterols (Rf =0.063, 0.344, & 0.875) were found during 9th, 10th and 11th day of germination. The pink band (Rf= 0.750) was not seen (Table 3).

These may be isomotiol, sitosterol, stigmasterol and compesterol or related sterols.

Alkaloids

Table 2: Qualitative analysis of alkaloids of *N. sativa* seeds during germination

S. No.	Colour of spot	Rf values	Days of germination											
			0	1	2	3	4	5	6	7	8	9	10	11
1	Greenish yellow	0.067	+	+	+	+	+	+	+	+	+	+	+	-
2	Greenish yellow(VL)	0.588	-	-	-	+	+	+	+	+	+	-	-	-
3	Orange	0.588	-	-	-	-	-	-	-	-	-	+	+	+
4	Greenish yellow	0.647	-	-	-	-	-	-	-	-	-	+	+	
5	Yellow	0.676	-	-	-	-	-	-	-	-	-	+	+	+
6	Pink(VL)	0.765	-	-	-	+	+	+	+	+	+	+	-	-
7	Brown(VL)	0.882	+	+	+	+	+	+	+	+	+	+	+	+

Sterols

Table 3: Qualitative analysis of sterols of *N. sativa* seeds during germination

S. No.	Colour of spot	Rf values	Days of germination											
			0	1	2	3	4	5	6	7	8	9	10	11
1	Brown(+++)	0.063	+	+	+	+	+	+	+	+	+	+	+	+
2	Brown(++)	0.344	+	+	+	+	+	+	+	+	+	+	+	+
3	Pink(++)	0.75	+	+	+	+	+	+	+	+	+	-	-	-
4	Brown(+)	0.875	+	+	+	+	+	+	+	+	+	+	+	+

Polyphenols

Table 4: Qualitative analysis of phenols of *N. sativa* seeds during germination

S. No.	Colour of spot	Rf values	Days of germination											
			0	1	2	3	4	5	6	7	8	9	10	11
1	Blue	0.04	+	+	+	+	+	+	+	+	+	+	+	+
2	Dark blue	0.067	+	+	+	+	+	+	+	+	+	+	+	+
3	Blue	0.1	+	-	-		-	-	+	+	+	+	+	+
4	Light blue	0.33	-	+	-		-	-	-	-	-	-	-	-
5	Blue	0.36	-	-	+	+	-	-	-	-	-	-	-	-
6	Blue	0.42	-	-	-	-	+	+	+	+	+	-	-	-
7	Intense blue	0.487	+	-	-	-	-	-	-	-	-	+	+	+

Study of phenolic contents of *N. sativa* by thin layer chromatography is tabulated in the Table 4. Seven different spots of phenols (Rf = 0.04, 0.067, 0.1, 0.33, 0.36, 0.42, 0.487) were reported during the germination of *N. sativa* seed. Further, it was observed that two spots (Rf =0.04, 0.067 were found to be common in all the germination stages. A spot (Rf = 0.33) was observed on 2nd day, a spot (Rf = 0.36) was observed on 3rd day, a spot (Rf = 0.42) from 5th to 8th day and a spot (Rf = 0.487) was seen from 9th to 11th day of germination. This might be due to the synthesis of new phenolic compounds during germination.

Flavonoids

Table 5: Qualitative analysis of flavonoids of *N. sativa* seeds during germination

S. No.	Colour of spot	Rf values	Days of germination											
			0	1	2	3	4	5	6	7	8	9	10	11
1	Green	0.125	+	+	+	+	+	+	+	+	+	-	-	-
2	Blue	0.31	-	-	-	-	-	-	-	-	-	-	-	+
3	Green	0.458	-	-	-	-	-	-	-	-	-	-	-	+
4	Blue	0.6	+	+	+	+	+	+	+	+	+	+	+	+
5	Blue	0.725	-	+	-	-	-	-	-	-	-	-	-	-

The data of thin layer chromatography of flavonoids of *N. sativa* is tabulated in the Table 5. Five flavonoid spots were reported in the different samples and they showed fluorescence of different colors under UV illumination on a transilluminator. In our observation, one spot (Rf = 0.6) was common in all the days of germination whereas a spot (Rf = 0.725) was seen only on first day when imbibitions took place by the seeds. Only single spot (Rf – 0.6) was present in the samples on 10th and 11th day of germination. It was interesting to see two new spots in the seedling sample (Rf = 0.31 & 0.458).

Cardiac Glycosides

Table 6: Qualitative analysis of glycosides of *N. sativa* seeds during germination

S. No.	Colour of spot	Rf values	Days of germination										
			0	1	2	3	4	5	6	7	8	9	10	11
1	Fluorescent Green	**0.031**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**+**	**+**	**+**	**+**
2	Fluorescent Green	0.046	+	-	-	-	+	+	-	-	-	-	-	-
3	Fluorescent Green	0.077	-	-	+	+	+	-	+	+	-	-	-	-
4	Fluorescent Green	0.108	-	-	+	-	-	-	-	-	-	-	-	-
5	Fluorescent Green	0.123	-	-	-	-	-	-	-	-	+	+	+	-
6	Fluorescent Green	0.154	+	-	-	+	+	+	-	+	-	-	-	-
7	Fluorescent Green	0.231	-	-	+	-	-	-	-	-	-	-	-	-

The data of cardiac glycosides of *N. sativa* by thin layer chromatography is tabulated in the Table 6. Seven different glycosides with similar green fluorescence were observed during germination of *N. sativa* seeds (Rf = 0.031, 0.046, 0.077, 0.108, 0.123, 0.154 & 0.231). A spot (Rf = 0.154) was reported in the samples of seed, 3rd, 4th and 6th day of germination, whereas a spot (Rf = 0.077) was present in 2nd to 7th day of germination. Two different spots (Rf = 0.108 & 0.231) were observed on 2nd day of germination and a band (Rf = 0.046) was present only on 4th day of germination. A spot (Rf = 0.123) was seen from 7th to 11th day of germination till the complete plantlet was formed which indicates the synthesis of this glycoside during later stages of germination whereas a band (Rf = 0.154) disappeared from these samples which was seen to be present during earlier germination stages (0, 3rd, 4th, 5th & 6th day).

Quantitative Estimation of Phytochemicals

Table 7: Quantitative estimation of phytochemicals of *N. sativa* seed during germination

Days	Total Phenols mg/g	Total Flavonoids mg/g
0	13.75±0.22	12.7±0.36
1	15.75±0.75	14.8±0.62
2	16.9±0.50	12.15±0.97
3	16.9±1.04	14.45±0.39
4	20.0±0.03	20.7±0.67
5	23.5±0.32	23.5±1.06
6	14.5±0.53	24.90±0.43
7	14.5±0.61	22.05±0.12
8	13.75±1.05	22.05±0.81
9	13.25±0.77	21.35±0.32
10	13.27±0.21	20.90±0.65
11	13.21±0.64	20.25±0.23

∗ The readings were taken in triplicate and values represent Mean ± SD**., *** The experiments were repeated thrice.

The phenols and flavonoids play an important role in giving protection to the plants against deleterious effects of UV rays and also against certain phytopathogenic microorganisms and are responsible for most of the medicinal properties of the plant. So present study also includes the estimation of total phenolic content and total flavonoid content of the extracts of various germination phases.

The data of quantitative estimation showed the presence of phenolic compounds and flavonoids in all the samples (Table 7). An overall increase in total phenols till 6th day was seen after which there was a little decline on 7th day, after which a continuous decrease in phenols was observed till the formation of seedling. A similar pattern was reported in flavonoid content also, larger concentration of both phenols and flavonoids was seen from 4th to 6th day of germination with a maximum reaching on 6th day. *N. sativa* contained the highest amount of phenols (23.5±0.32 mg/g of fresh weight) on 5th day, and flavonoids (24.9±0.43 mg/g of fresh weight) on 6th day of germination. The seedling was shown to have least content of these two phytochemicals. This might be due to the consumption and biochemical modification of stored metabolites during germination as the mode of nutrition changes from the heterotrophic to autotrophic mode.

DISCUSSION

The medicinal properties of plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*. The successive extracts of seeds of *N. sativa* in different phases of seed germination have revealed the presence of alkaloids, sterols, flavonoids, phenols, tannins, saponins, terpenoids and glycosides (Table 1). Alkaloids are the lead molecules of therapeutic importance from *N. sativa.* These are heterocyclic indole compounds which have proved to be have pharmacological properties such as antifungal activity [18], hepatoprotective, antimicrobial and antimalarial activities [19].The presence of alkaloids in most of these samples supported the reports of various authors [20]. The decrease in alkaloid content, during the early hydrolytic phase of germination and the subsequent increase during the early synthesis phase, follows the pattern of the primary food reserve in the seed and indicates that alkaloids are readily involved in metabolism and are probably not metabolic end products or waste products as often suggested [21,22]. The results of the study revealed the presence of sterols in all the phases of germination. These result is coincides with the other studies which reported that the total, free, and esterified sterols increased, with stigmasterol and campesterol while steryl glycosides decreased. During germination, sitosterol was the major sterol in all fractions [23].The plant sterols help in the lowering plasma cholesterol and LDB cholesterol. Hence, its inclusion in diet will be helpful in preventing cardiovascular disease. Another point of concern is that plant sterols could act as a natural preventive dietary product [24]. A similar pattern was reported in phenols and flavonoid content; larger concentration of both phenols and flavonoids was seen from 4th to 6th day of germination with a maximum reaching on 6th day. The seedling was shown to have least content of these two phytochemicals. This might be due to the fact that the stored metabolites in the seeds have been utilized during germination and therefore the fresh synthesis of these compounds have not been started till the plant switches over completely from the heterotrophic mode of nutrition to the autotrophic mode. These

compounds were found to be toxic to the growth and development of pathogens [25]. Presence of tannins in the later stages of germination suggests the ability of these plant samples to play a major role as antidiarrhoea and antihaemorrhagic agent [26, 27]. The oxidation inhibiting activity of tannins have been known for a long time and it is assumed to be due to the presence of gallic and diagallic acids. Another point of note in this study is the styptic and stringer properties of tannic acid which was used in the treatment of inflammatory skin eruption and bowel conditions. The presence of tannins is also responsible for the astringent flavor of *N. sativa* seeds. The occurrence of steroidal saponins from numerous studies showed their importance and interest in pharmacy due to relationship with such compounds as sex hormones mostly in the development of female contraceptive pills. Additionally, saponin is equally used in medicine and pharmaceutical industries because of its foaming ability with the production of frothy effect. Saponin is used in the preparation of insecticides, various drugs and synthesis of steroid hormones [28]. This compound has also been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties [29, 10]. Cardiac glycosides showed positive results in the initial stages of seed germination, the colour intensity decreased as the germination proceeds. The cardiac glycosides have been used for over two centuries as stimulants in cases of cardiac failure [30]. This perhaps justifies the already locally established function of the seeds of this plant in the treatment and management of hypertension.

CONCLUSION

The preliminary screening tests facilitate quantitative estimation and qualitative separation of pharmacologically active compounds and subsequently may lead to the discovery and development of novel drugs. From the above analysis it could be concluded that an overall decrease in alkaloids, saponins, and glycoside contents was observed during germination, while there was an increase in phenols, tannins and flavonoid contents. Phytochemical screening of extracts of samples of different had revealed the presence of flavonoids, tannins, terpenoids, saponins, steroids, alkaloids in the 5^{th} and 6^{th} days germinated seeds by positive reaction with the respective test reagent. Hence, the 5^{th} /6^{th} day germinated seeds of *N. sativa* can be seen as a potential source of drugs.

The data generated from these experiments have provided the chemical basis for the wide use of this plant in germinating stages as therapeutic agent for treating various ailments. Further investigation on the isolation and characterization of these phytochemicals is however required.

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