RELEVANCE OF ADJOINING NIGELLA SATIVA SEEDS IN A COMMERCIALIZED HERBAL TEA POWDER

Comparative study of commercially available herbal tea before and after addition of black cumin seeds

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Abstract: The plant *Camellia sinensis* yields a variety of white, green and black tea. Tea is one of the most widely consumed beverages in the world, next to water for enjoyment and health. In the present study, the relevance of addition of 0.2% of aqueous extract of *Nigella sativa* (black cumin seeds) to a herbal tea powder (containing 20 g each of *Zingiber officinale* (Ginger) and seeds of *Phoenix dactylifera L* (Ajwa seeds in 100 g of tea powder) was analyzed by means of analyses like different phytochemical compound screening, calculation of antioxidant activities and nutritive value determination. The above mentioned assays were used for the comparison of herbal tea before and after addition of appropriate amount of black cumin seeds. Phenol content was increased from 143.66 ± 2.02 mg GAE/g to 280.22 ± 1.03 mg GAE/g and flavanoid content was raised from 1.17 ± 0.10 mg QE/g to 3.9 ± 0.35 mg QE/g on inclusion of black cumin seed extract. There was a twofold increase for antioxidant activity from 5.73 ± 0.51 mg/mL to 12 ± 1.12 mg/mL. The nutritive value was also raised from 325.96 K Cal/ 100 g to 410.11 K Cal/ 100 g. The findings of the study revealed that *N. sativa* seeds has potential phytoconstituents which can trigger biological activities, therefore these experimental seeds can be further included into tea powders for pharmacological preparations. The overall analyses pave the way of prospect for the addition of black cumin seeds to normal herbal tea powders commercially available in the present market as a remedy for various microbial diseases in humans. In the present scenario of pandemic COVID 19, this final tea product can act as an immunity booster by incorporating them in our daily routine which can be proved by assessing further detailed characterization in future.

***Keywords: Camelia sinensis, Nigella sativa,* black cumin seeds, *Zingiber officinale, Phoenix dactylifera L,* Phytochemical, Antioxidant, Nutritive value**

I. INTRODUCTION

Free radicals refer to a set of compounds with elevated reactivity by reason of impaired electrons in the outer orbital. Reactive oxygen species (ROS), to name a few, superoxide anion, hydroxyl radical and hydrogen peroxide, as well as reactive nitrogen (RNS) species, including nitric oxide and peroxynitrite, belong to this group. Whilst of structural distinction, free radicals share common mechanisms that affect the cells and tissues of the body by protein, DNA and lipid damage. An important, radical species-dependent damage, either when contemplating the organism as a whole or a particular incorporated function, such as the immune response, is the alteration of membrane functions occurring consequently of phospholipids changes. Potential therapeutic uses of antioxidants in free-radicals, have led to the hypothesis that, their use may slow down or reverse symptoms associated with neurodegenerative disorders or other related diseases. As any other animal body, human body also has its own antioxidant system which is made up of enzymes such as catalases, superoxide dismutases and metal-binding proteins. Cellular antioxidant defense system is endogenously activated by glutathione and other enzymes that transform the oxidized molecules into their own reduced form. The endogenic defense system is exacerbated by antioxidants such as Vitamin C, vitamin E, carotenoids, flavonoids,which are mainly found in vegetables, fruit and green tea (Korany and Ezzat, 2011).

Plants play an important role in global health. They have the ability to synthesize numerous organic compounds that are considered to be secondary metabolites. The fundamental properties of plant secondary metabolites are their distinct carbon skeleton structures. Secondary metabolites are not required for a cell to survive, but play a role in the cell's relationship with its environment, maintaining the organism's continued survival. Secondary metabolite production is typically unique to the organ, tissue, and cell, and these are compounds of low molecular weight. In terms of their number and quantities, these compounds also vary between individuals from

the same population of plants. Secondary metabolites shield plants from, both biotic (bacteria, fungi, nematodes, insects or animal grazing) and abiotic (higher temperature and humidity, shading, heavy metal damage or presence) stresses. Due to a high economic benefit, secondary metabolites are used as chemicals in particular such as additives, fragrances, insecticides, and dyes. Furthermore they are considered to be the most important source of natural medicines. Plants have been used as medicine by almost all civilizations from ancient times. World Health Organization (WHO) has recently reported that majority of the people prefers medicinal plants for their primary health care (Yessuf, 2015). Based on their novel modes of pharmacological action, natural products obtained from medicinal plants may be the basis for looking for new medications.

Tea, a drink made from the leaf and bud of the *Camellia sinensis* plant, is the commonly drunk beverage in the world. Polyphenols are the most interesting category of green tea leaf ingredients and as a result, green tea can be regarded as an essential dietary source of polyphenols, flavonoids. Catechins are the major components of green tea extract and are highly potent flavonoids contained in tea. Catechins can serve as the best dietary natural antioxidants sources. Catechins function as free radical scavengers that provide chemo-preventative actions as well as defense against coronary heart disease and high blood pressure attenuation (Negishi *et al*., 2004).

Black cumin *(Nigella sativa)* belongs to *Ranunnclaceae* family and is declared worldwide as a medicinal spice. The plant grows in the regions of Mediterranean and Asian countries, such as India, Pakistan , Indonesia, Italy and Afghanistan. Since the seeds contain phenolics, flavonoids , tannins etc, there is a strong possibility for using them as sources of natural antioxidants. Phytochemically and pharmacologically, the *Nigella* seeds have been studied most extensively (Desai *et al*., 2015). It has been shown that the oil and aqueous extracts of *N. sativa* seeds have antioxidant, anti-inflammatory, anti-cancer, analgesic and antimicrobial potential. Additionally, phenolic compounds are shown to be capable of regenerating alpha tocopherol into its active antioxidant type. So the seeds have been used for medicinal purposes for decades. It's named 'Panacea' in old Latin, meaning 'cure everything'.Naturally, these phytochemicals appear in almost all plant materials, prominently in spices, as secondary metabolites. Owing to their free radical scavenging and metal chelating activities, it has beneficial roles as antioxidants in human health such as cancer care and prevention, cardiovascular disease other pathological conditions. Recent research in food phenolics has grown significantly as these natural antioxidants encourage in the prevention of toxicity disorders.

Most people, nowadays, are aware that, it is not exactly a tea, but the finely ground mix of herbs and leaves mixed with leaves of tea plants make them attractive. Tea can be made as hot drink with black seeds or oils like that of black cumin seeds, which in line boosts the antioxidant property and gaining attention recently throughout the world. Though raw seeds have a strong taste, their tea equivalent is reminiscent of chamomile with a very mild flavor profile with enhanced health benefits.

Ginger has been mainly exploited as a food seasoning ingredient and medicinal resource particularly for treating diseases related to inflammation and oxidative stress (Shareef *et al*., 2016). For its extensive range of health functionality, the presence of higher concentration of phenolic derivatives plays an important role (Tohma *et al*., 2017). Over the most recent three decades, widespread studies have been conducted to understand the detailed chemical composition and biological activities of normal ginger. Phenolic acids, diarylheptanoids, terpenoids, and flavonoids were reported to exist in ginger rhizomes. Ajwa date seeds are very healthy, especially known and proven to have immense effects on the cardiovascular system. It is loaded with all the essential elements like Iron, Calcium, Potassium, Zinc minerals, vitamin B-12 and Biotin which is extremely helpful in reducing cholesterol and fat levels. Ajwa dates seed powder is highly effective for the treatment of blocked heart arteries. It helps to reduce triglycerides and cholesterol levels (LDL) in the blood.
Because of carbohydrate content, Ajwa dates also provide a burst of energy that leads to a good exercise routine and which in turn helps improving heart health.
It has been advised after bypass surgery or angioplasty. People who have high blood pressure problem and smokers, should take ajwa seed powder daily in the morning. It is also useful in male and female infertility (Khalid *et al*., 2017).

An assortment of randomized controlled trials, pilot studies, case reports and in vitro and in vivo studies corroborated that *N. sativa* has antiviral, anti-inflammatory, antioxidant, immunomodulatory, antihistaminic, bronchodilatory, antitussive activities related to causative oraganism and signs and symptoms of COVID-19. Additionally, *N. sativa* has also shown anti-hypertensive, anti-obesity, anti-diabetic, anti-hyperlipidemic, anti-ulcer, and antineoplastic activities which would help the COVID-19 patients with comorbid conditions. Besides, the active constituents of *N. sativa* such as nigellidine and α-hederin have been identified as potential inhibitor of SARS CoV-2 (Maideen, 2020).

Thus the present research is aimed to assess the proximate contents of a herbal tea powder containing 20 g/wt each of *Zingiber officinale* (Ginger) and seeds of *Phoenix dactylifera L* (Ajwa seeds) and then find the relevance of adding *Nigella sativa* (black cumin seeds) (20 g/wt) to strengthen the flavor as well as antioxidant capability of tea powder after needful analyses.

II. MATERIALS AND METHODS

2.1. Analysis of tea powder

2.1.1. Proximate analyses

Proximate analyses were done using the method of Association of Official Analytical Chemists (AOAC., 2005).

2.1.1.1. Moisture Content Determination

Petri dishes were selected, cleaned and labeled. The dishes were dried in a hot air oven at 105ºC for about 30 minutes and cooled in an air-tight desiccators. The dishes were then weighed after being removed from the desiccators using a calibrated digital analytical balance and their various weights were recorded. 2g of each sample was weighed into the labeled pre-weighed Petri dishes and weighed again. They were immediately transferred into an air oven pre-set at 105ºC to dry. Samples in crucibles had their weights measured severally until they obtained constant weights as final values. The difference in initial and final weights indicated moisture contents of tea samples.

% Moisture = (Total weight – Final weight) / Weight of the sample x 100

2.1.1.2. Ash Content Determination

Porcelain crucibles were selected (3 for each sample), cleaned and labeled. The crucibles were dried in the hot air oven at 105ºC for about 30 minutes and placed in a desiccator to cool. The initial weights of the cooled crucibles were recorded. 2.0g of each sample was weighed into each of the crucibles and placed in a muffle furnace (Gallenkamp model) to carbonize at 600ºC for 2 hours till the samples had a cotton wool like texture. The crucibles with the ash were removed and kept in a desiccator to cool. They were reweighed and the percentage ash was calculated.

% Ash = Weight of ashed sample / Weight of sample taken x 100

2.1.1.3. Fat Content Determination

1.0g each of the samples was weighed into a pre-weighed thimble. 150ml pet ether was measured into a 250ml conical flask using the measuring cylinder. The samples were then extracted under reflux using a soxhlet extractor fitted with thimble containing samples. The extraction was stopped after eight (8) hours and the thimbles with contents were removed, dried in an oven at 105ºC for two hours and weighed with an analytical balance.

% Crude fat = (W2 - W1) / Fresh sample weight x 100

2.1.1.4. Protein Content Determination

One gram (1.0g) each of the samples (tea formulation A and Lipton) was weighed into the kjeldahl flask. 1.0g of K_2SO_4 and 0.1g Ca_2SO_4 were added into the flask and mixed with 20ml of H_2SO_4 after which the flask was placed on the kjeldahl digestion heating mantle in a slanting position in a fume chamber. The process was monitored until a color change which was from black to bluish-green was observed. The reaction was then stopped and digests removed and cooled. They were then topped with water and made up to 200ml mark on ice. 50ml of aliquot of each digest was poured into a distillation flask and carefully layered with 50ml of NaOH. The solution was then distilled into a receiving flask containing 50ml of 0.1N H_2SO_4 with two drops of methyl red as an indicator. Distillation was stopped by removing the solution in the receiving flask immediately before putting of hot mantle to avoid drop in pressure. The distillate was titrated with 0.1M NaOH and percentage nitrogen calculated as protein content.

2.1.1.5. Fiber content determination

Weigh accurately about 2 - 2.5 gm ground sample into a thimble and extract for about 1 hour with petroleum ether in a soxhlet extractor. Transfer the material in the thimble to a 1 litre flask. Take 200 ml of dilute sulphuric acid in a beaker and bring it to boil. Transfer the whole of the boiling acid to the flask containing fat free material and immediately connect the flask to a water cooled reflux condenser and heat, so that the contents of the flask begin to boil within 1 minute. Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid. Continue boiling for exactly 30 minutes. Remove the flask and filter through fine linen (about 18 threads to a cm) or through a coarse acid washed , hardened filter paper held in a funnel and wash with boiling water until the washings are no longer acid to litmus paper. Bring some quantity of sodium hydroxide solution to boil under a reflux condenser. Transfer the residue on the filter into the flask with 200 ml of boiling sodium hydroxide solution. Immediately connect the flask with the reflux condenser and boil for exactly 30 minutes. Remove the flask and immediately filter through the linen or filter paper. Thoroughly wash the residue with hot water and transfer to a gooch crucible prepared with a thin but compact layer of asbestos. Wash the residue thoroughly first with hot water and then with about 15 ml of ethanol and with 3 successive washings of petroleum ether. Dry the gooch crucible and contents in an air oven at 105±1°C for 3 hours. Cool and weigh. Repeat the process of drying for 30 minutes, cooling and weighing until the difference between two consecutive weighings is less than 1mg. Incinerate the contents of the gooch in a muffle furnace at 550±25° C until all carbonaceous matter is burnt. Cool the gooch crucible in a desiccator and weigh.

Crude fiber percent by weight= (W1-W2/W) * 100/(100-M)*100

Where,

W1= Weight of gooch crucible+contents+asbestos before ashing

W2=Weight of gooch crucible+ ash and asbestos after ashing

W=Weight of sample taken for test

M=Percent moisture content

2.1.1.6. Determination of carbohydrate

Carbohydrates are calculated based on the following tests and their respective method references: Moisture (method is matrix dependent), Ash (AOAC 923.03), Fat by gas chromatography (AOAC 996.06) or by hydrolysis/extraction (AOAC 933.05; 922.06; 948.15; 925.32; 950.54; 905.02; 920.111; 945.48G; 920.115F; 932.06; 920.09; 952.06), Protein (AOAC 968.06) and, optionally, Insoluble and Soluble Dietary Fiber (AOAC 991.43 or 2011.25) and Sugar Alcohols (internally developed method).

Method Description: Carbohydrates are determined by the following calculation: Carbohydrates = 100 – (% Ash) – (% Total Fat) – (% Moisture) – (% Protein)

2.1.1.7. Determination of Nutritive value

The nutritional value was calculated as per the formula used by Nile and Khobragade (2009). Nutritive value = (4 x percentage of protein) + (9 x percentage of fat) + (4 x percentage of carbohydrate)

2.1.1.8. Water Extractives Determination

Beside proximate analysis, the amount of solids that can be extracted from tea samples when brewed (water extractives) was determined for both tea samples. 2.0g of each sample was weighed into round bottom flasks and fitted to reflux condensers. 100ml distilled water was added to each of them and were boiled for one hour and filtered using Whattman filter paper. The filtrates were poured into beakers and evaporated over steam on a water bath until the contents on the beakers reduced by about 95%. At this time, the beakers were removed into a hot air oven to dry. The beakers containing the dry sample solids were removed after drying and placed in a desiccator to cool. They were then reweighed and their respective weights recorded. The percentage total solids were calculated for the two tea samples.

2.1.2. Estimation of Iron

Iron content was estimated by the method of Thomas, R. A and Krishnakumari, S. (2015). Taken 1.5 ml of extracted seed sample and 1.5 ml of prepared ash solution in test tubes, added 1.0 ml of 30% H_2SO_4 and 1.0 ml of 7% potassium per sulphate solution and 1.5 ml of 40% potassium thiocyanate solution are added. The red color developed was read at 540 nm within 20 minutes. The standard aliquots with concentration corresponding to 10- 50 µg were treated similarly. The estimation was done in triplicates and the results were expressed mg/g sample.

2.2. Analyses of *Nigella sativa* seeds

2.2.1. Sample preparation

Black cumin seeds were purchased and were stored in the laboratory conditions. Powdered samples of each type were prepared by grinding and ground samples were kept in glass containers separately for subsequence used in the study. Extraction process was carried out by adding 10g of powder into 100ml of water and was kept on shaker overnight. Then extracts were centrifuged 1100× for 10 minutes, followed by filtration by using a Whatman grade 1 qualitative filter paper and solutions were stored in brown bottles at 4 °C (Thilakarathne *et al*.,2018).

2.2.2. Phytochemical Screening

The crude extract was tested for the presence of bioactive compounds by using following standard methods (Hamburger and Hostettmann.,1991), (Kamal.,2014) , (Yessuf.,2015) and (Madhukar.,2013).

2.2.2.1. Qualitative screening

2.2.2.1.1. Alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's test: Mayer's and Wagner's reagents were then added to mixture, resulting orange precipitate was taken as evidence for presence of alkaloids.

2.2.2.1.2. Saponins

Froth's test: Crude extract was mixed with 5 ml of distilled water in a test tube and shaken vigorously then was left to stand for 10 minutes and the result was noted. No thick persistent froth was observed. This indicated the absence of saponins.

2.2.2.1.3. Phenol

Ferric chloride test: 2ml of extract mixed with 5% ferric chloride solution and result was noted. Deep blue black colour indicated the presence of phenol.

2.2.2.1.4. Tannin

Some amount of extract was dissolved in distilled water and then 2ml of ferric chloride was added in the solution. Formation of blue green colour indicated presence of tannin.

2.2.2.1.5. Flavonoids

Alkaline reagent test: Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turns colourless on addition of few drops of diluted acid which indicated the presence of flavonoids. To the test solution, few drops of ferric chloride solution was added, green colour indicated the presence of flavonoids. Crude extract was mixed with few fragment of magnesium ribbon. Then concentrate HCl was added drop wise. The development of pink scarlet coloration after few minutes indicated the presence of flavonoids.

2.2.2.1.6. Sterol

Extract was mixed with 2 ml chloroform and H_2SO_4 was added side wise. A red colour produced in lower layer of chloroform that indicated the presence of steroids.

2ml of extract mixed with 5ml of chloroform and 2ml acetic anhydride was added followed by concentrated H_2SO_4 and result was noted. Reddish brown colour indicated the presence of steroids.

2.2.2.1.7. Terpenoids

Extract was taken in chloroform with few drops of concentrate sulphuric acid, shaken well and allowed to stand for some time. Formation of yellow colour layer indicated the presence of terpenoids.

2.2.2.1.8. Glycosides

Extract hydrolysed with concentrate HCl for 2 hours on water bath, filtered and hydro lysate was subjected to following test. Borntrager's test: To 2ml of filtered hydro lysate, 3ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. The pink colouration indicated the presence of glycosides.

2.2.2.1.9. Protein

Millon's test: Crude extract was mixed with 2ml of millon's reagents and result was noted. White precipitated which turned red upon gentle heating that confirmed the presence of protein.

2.2.2.1.10. Carbohydrates

Molish test: Extract mixed with 2ml of molish reagent and mixture was shaken and poured carefully along the side of test tube. Appear violate ring at interphase indicated the presence of carbohydrate.

2.2.2.2. Quantitative screening

2.2.2.2.1. Determination of phenols

Using a pipette, transfer 1,0 ml of the sample extract into a one-mark 100 ml volumetric flask. Dilute to the mark with water and mix.Using a pipette, transfer 1,0 ml of the gallic acid standard solutions A, B, C, D and E in duplicate into separate plastic or graduated tubes .These correspond to approximately 10 µg, 20 µg, 30 µg, 40 µg and 50 µg of anhydrous gallic acid. Using a pipette, transfer 1,0 ml of water, in duplicate, into separate tubes. These are reagent blanks. Using a pipette, transfer 1,0 ml of diluted sample extract, in duplicate, into separate tubes.Using a pipette, add 5,0 ml of dilute Folin-Ciocalteu phenol reagent into each tube and mix.Within 3 min to 8 min after the addition of the dilute Folin-Ciocalteu phenol reagent, pipette 4,0 ml of sodium carbonate solution into each tube. Stopper and mix.Allow to stand at room temperature for 60 min, and then measure the optical densities in 10-mm path length cells against water on the spectrophotometer set at 765 nm.The reagents blank should have an optical density of < 0.010. Higher values indicate contamination problems from poor quality water, reagents or glassware. It is also important that the sample

optical density be within the calibration range. Repeat the colorimetric assay with an increased dilution if the sample optical density is higher than the 50 µg gallic acid standard E (Ahmed *et al*.,2018).
Calculate, to the nearest 0,1 µg, the mass of anhydrous gallic acid, m, in the 1,0 ml aliquots of thestandard solutions A, B, C, D and E using the formula:

$m = m_0 * V * W_{DM\ std} * 10000/(100 * 100)$

m0 is the mass of gallic acid monohydrate, in grams, used to prepare the stock standard solution

V is the volume of gallic acid stock standard solution, in millilitres, used to prepare the standard solutions A, B, C, D and E
wDM, std is the dry matter content, expressed as a mass fraction, in percent, of the gallic acid.

2.2.2.2.2. Determination of total flavonoid content
Aluminum chloride method was used for flavonoid determination. In this method Quercetin was used as standard and flavonoid content were measured as quercetin equivalent. An aliquot (0.5ml) of extract were taken and different concentrations(10 -50 microgram/ml) Of standards was taken into 10ml volumetric flask , containing 4ml distilled water and 0.3ml of 5% $NaNO_2$ were added to the flask. After 5 min, 0.3ml of 10% $AlCl_3$ was added to the mixture. At the 6^{th} min, 2ml of 1M NaOH was added and volume was made up to 10 ml with distilled water. After 15 min of incubation the mixture turned to pink, the absorbance was noted at 510nm using UV-Visible spectrophotometer. Distilled water was used as blank.The TFC was expressed in mg of Quercetin equivalents per gram of extract (Ahmed *et al*.,2018).

2.2.3. Total Antioxidant Activity

Total antioxidant activity of the extract was determined according to the method of Ahmad *et al*.,2013. 0.3mL of each fraction was mixed with 3.0mL of reagent solution(0.6M sulphuric acid, 28mMsodium phosphate, and 4 mM ammonium molybdate). Reaction mixture was incubated at 95∘C for 90 min in a water bath. Absorbance of all the sample mixtures was measured at 695 nm. Ascorbic acid (100 *µ*g/mL) was used as standard control.

2.3. Analysis of *Zingiber officinale* (Ginger) and seeds of *Phoenix dactylifera L* (Ajwa seeds)

Ginger (20 g in 100g of tea powder) and ajwa seeds (20 g in 100 g of tea powder) are the inherent components of the herbal tea powder used for the assay. They were incorporated to enhance the flavor and medicinal properties of tea powder. Thus the analyses (every tests done for *Nigella sativa* seeds) for both ingredients need to be assayed for the final comparison of herbal tea powder after incorporation with processed black cumin (*N. sativa*) seeds (20 g/wt).
Assays were repeated in same procedures as per section 2.2.

2.4. Comparison of herbal tea powder before and after addition of *Nigella sativa* seeds

Phenolic and flavanoid contents along with the antioxidant activities and nutritive values of herbal tea powder before and after addition of 0.2 % of black cumin seed extract were done to prove the relevance of new component.

III. RESULTS AND DISCUSSION

3.1. Analyses of tea powder

3.1.1. Proximate Analysis

The nutritive quality of food materials can be evaluated by analyzing its proximate composition. The quantitative analysis of moisture, ash content, crude fat, crude fibre, carbohydrate and protein were done to find out the nutritional properties of the tea powder sample. The assays are done in triplicates and values are expressed by mean ± SD. The results are given in the **figure 1** and the proximate composition is expressed as a measure of percentage (%) in the sample.
The nutritive value was calculated to be 325.96 K Cal/ 100 g for the tea powder sample.

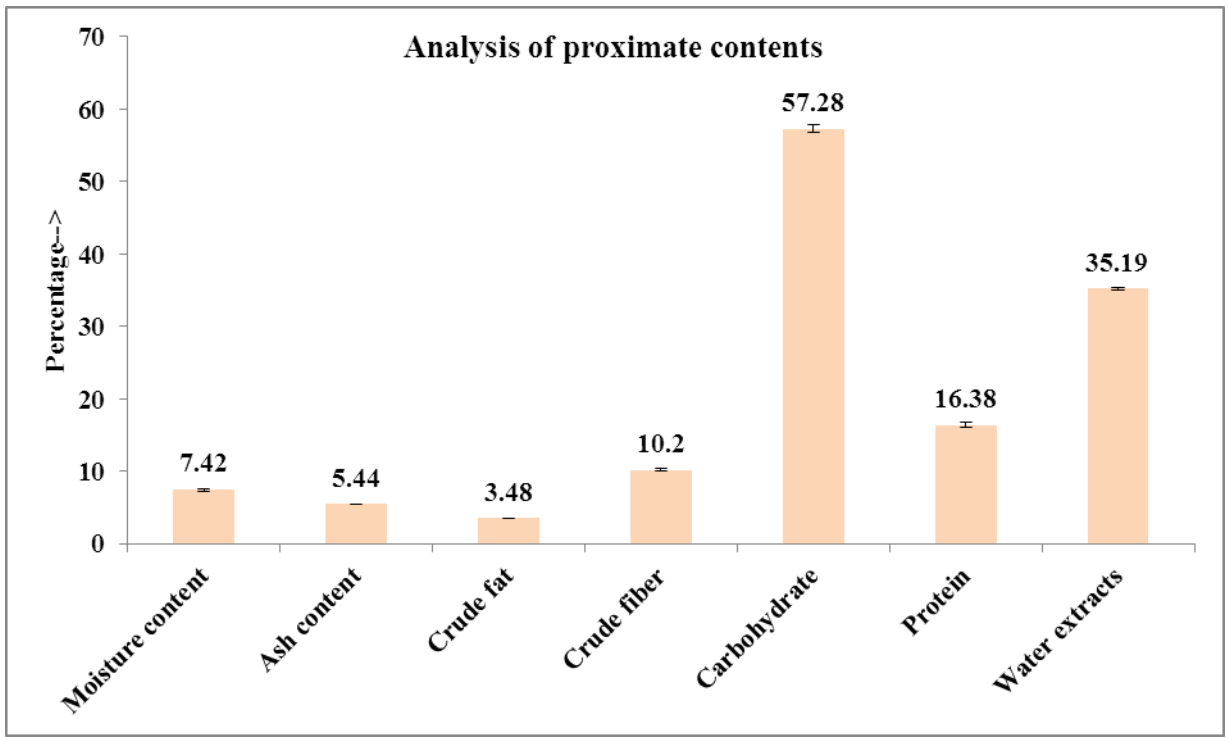

Fig. 1: Analysis of nutritive contents

Plants are the rich source of all the components that are crucial for human beings. The usage of therapeutics of medical plants as supplements in diet has risen significantly in the last decades. The new research gives a detailed overview of nutritive parameters of tea powder. The proximate analysis as well as the mineral elements such as iron may benefit in the detection of the dietary elements that are bioactive in nature as well as responsible for the therapeutic properties of tea powder. Ash comprises inorganic material because the process of ashing rescinds all the other organic material existing in the sample. Ash also reflects high digestibility of the plant (Ibrahim *et al*., 2010). Ash content and moisture is highly correlated .Ash content of tea powder is also considered as an important parameter of quality. The higher ash content in tea powder is due to low moisture content. Previous researchers also indicated positive relationship between ash content and keeping quality of tea and proposed that ash content should be controlled less than 5.54% in order to maintain quality of tea during storage (Ismail *et al*., 2000; Rehman *et al*., 2002)..

Fiber content can be of concern to human health since the fibrous is readily digested and disintegrated. For digestion and the successful removal of waste, fibres in the diet are important (Hussain *et al*.,2011). High fiber content can decrease serum cholesterol, coronary heart disease risk, asthma, constipation, diabetes and cancer of the colon and breast (Hussain *et al*., 2009).The results in the present study revealed the presence of moderate amount of fiber content.When the impurities such as stem are used during the processing fiber content will decrease. Moreover, crushing, tearing as well as cutting processes csn also destroy the leaf structure that might have negative effect in the fibre content (Śmiechowska and Dmowski.,2006). At the same time higher amount of fat contents in tea may indicate absence of fermentation during processing (Rehman *et al*., 2002).

The higher moisture content in the tea samples may be due to exclusion of fermentation process.During processing of tea powder polyphenols may get destroyed and that may have retained the moisture content. The other important factor is the packaging material used.Inorder to maintain a constant moisture level during storage of commercial tea powder, appropriate packaging material should be selected. According to Venkatesan *et al*., 2006, higher moisture percentage (8%) can have negative effect on shelf life, so for the better quality of the product moisture percentage should be controlled between 2.5-6.5%.

The protein content was relatively higher in the present study (16.38%), but the results only showed protein contents of raw tea powder but did not in infused tea. This should be addressed in future studies as high protein drinks may pose health risks to certain individuals beside the fact that they are helpful in weight loss and muscle building (Jeor.,2001). Water extract of tea is reliant on various components such as sugars, phenols, alkaloids, amino acids and other soluble substances, like minerals and pigments. According to international standards it should not be less than 32% of the dry mass basis. This was partly due to the smaller granular size of tea contents which gave it a larger surface area, enabling faster and easier dissolution in hot water (Martinez and Amidon.,2002). The results of the present study are in line with the findings of Yao *et al*. (2006) who observed water extract of different types of tea to be in the range of 35.42%- 39.18%.

3.1.2. Analysis of iron content

In the present study, the amount of iron fillings in the tea powder sample was found out to be 247 ± 2.1mg/kg. Qualitative as well as quantitative determination of mineral elements in plants is vital. Mineral elements are desirable in miniscule quantities for the proper functioning of the human system functioning, health growth and moreover development (Igwenyi *et al*., 2014). The presence mineral elements in plants depend to a high degree on the soils abundance. Iron is considered to be the most important mineral in biological system. It implements an array of biological functions. Iron has a unique role in the process of human metabolism. In humans and

animals, iron is associated with hemoglobin and the oxygen transfer from lungs to the tissue cells. Iron deficiency is the most prevalent nutritional deficiency in humans. Iron also assists the oxidation of fat, protein and carbohydrates to control body weight, which in turn helps in diabetes (Hannah and Krishnakumari., 2005).

3.2. Analyses of *N. sativa* (black cumin) seed powder, *Z. officinale* (Ginger) and seeds of *Phoenix dactylifera L* (Ajwa seeds)

3.2.1. Sample collection and extraction

Black cumin seed were successfully extracted using water as solvent. The extract preparation was done the method of maceration. Maceration is a method that is suitable for compounds that do not withstand heating at high temperatures (Ramaa *et al*., 2006). In maceration, based on the principle of mass transfer of substance the chemical components will get attracted. The movement of the chemical compounds began to occur at the interface layer and later it diffuses into the solvent (Sethi *et al*., 2008). Many studies have reported that higher extraction yield in polar solvents (Rader *et al*., 2007; Hadi *et al*.,2016; Truong *et al*.,2019). This could be due to the presence of polar compounds in plants, that are soluble in solvents with high polarity such as water, methanol, and ethanol. In order to better understand the solvents effect on extraction yield, further analysis must be performed to measure the content of bioactive compounds in the extract. **Figure 2** demonstrates the extraction of black cumin seeds.

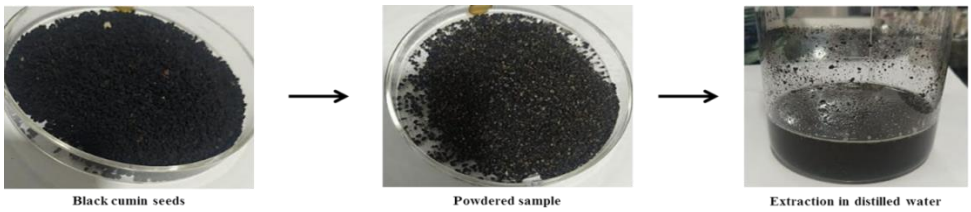

Fig. 2: Extraction of Black cumin seeds

Ginger and ajwa seeds were processed according and extracted in the same pattern as that of black cumin seeds to obtain the aqueous extracts.

3.2.2. Phytochemical screening

3.2.2.1. Qualitative analysis

The phytochemical screening of aqueous extracts of *N.sativa* seeds, *Z.officinale and Phoenix dactylifera* seeds revealed the presence of important phytocomponents. These phytochemical compounds are recognized to have fruitful importance in medicinal and moreover physiological activities. The extraction and identification of such bioactive compounds, can lead to the formulation of new drugs against various diseases as well as disorders. Alkaloids are a large group of compounds which are nitrogenous and are used widely as agents for cancer chemotherapeutic (Reddy *et al*., 2018). Flavonoids are one of the other phytocompound that wield an eclectic biological activities such as antimicrobial, anti-inflammatory, antioxidant etc (Kumar *et al*., 2010). One of the other ubiquitous groups of plant metabolites are phenolic compounds. Phenols possess biological properties including anti-oxidant, anti-ageing,anti-inflammation, endothelial functional improvement, angiogenesis inhibition as well as activities in cell proliferation (Han *et al*., 2007).Tannins are used medicinally as anti-haemorrhoidal compounds as well antioxidant (Nascimento *et al*., 2000) are also reported to found in aqueous extract of *Nigella sativa* . Glycosides are known as the blood pressure agent as per many reports (Reddy *et al*., 2018).Protein and carbohydrate are also considered to be important metabolites. Table 1 shows the results of phytochemical screening of all the three components.

Table 1. Showing the results of phytochemical screening

Phytochemicals	Black cumin extract	*Zingiber officinale*	*Ajwa seeds*
Alkaloid	+	+	+
Saponin	-	-	+
Phenol	+	+	+
Tannin	+	-	-
Flavonoid	+	+	+
Sterol	-	-	-
Terpenoid	-	+	-
Glycosides	+	+	-
Protein	+	+	+
Carbohydrate	+	+	+

3.2.2.2. Quantitative analysis

3.2.2.2.1. Estimation of total phenolic content

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate, inhibit lipooxygenase and scavenge free radicals. The amount of total phenol was determined with Folin – Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed mg gallic acid equivalence/g gallic acid equivalent using the standard curve : $y = 0.0094x + 0.0187$ and $R^2 : 0.9936$.

Table 2 illustrates the phenol contents of all the above mentioned components in mg GAE/g.

Table 2. Phenol content

Component	**Phenol content (in mg GAE/g)**
Nigella sativa	11.62 ±0.73
Zingiber officinale	5.04±0.12
Ajwa seeds	3.11±0.72

3.2.2.2.1. Estimation of total flavonoid content

The structural differences in flavonoid as well as their substitutions sway the stability of phenoxyl radical and thereby affect the antioxidant properties of flavonoids (Wojdyło *et al*., 2007). The amount of total flavonoid was determined with the Quercetin reagent. Quercetin was used as a standard compound and the total flavonoid were expressed as mg quercetin equivalent/g using standard curve equation: $y = 0.0873x - 0.0088$ and $R^2 = 0.9916$.

Table 3 illustrates the flavanoid contents of all the above mentioned components in mg QE/g.

Table 3: Flavonoid content

Component	**Flavanoid content (in mg QE/g)**
Nigella sativa	5.04 ±0.060
Zingiber officinale	1.36 ± 0.081
Ajwa seeds	1.02± 0.075

3.2.3. Antioxidant activity

N. sativa seeds are considered as a natural remedy for a variety of disorders including liver diseases. The hepato-protective effects have been found to be strongly related to its antioxidant potentials. The mechanism of action of free radical scavengers involves membrane stabilization, free radical neutralization as well as immune-modulation.The decrease in absorbance at 695 nm of Phosphomolybdate solution is referred to a standard curve obtained by using known concentrations of Ascorbic acid solution. The standard curve obtained was $y = 0.0735x + 0.1387$.

Table 4 illustrates the total antioxidant activity of all the above mentioned components in mg/mL.

Table 4: Total antioxidant activity

Component	**Antioxidant Activity (in mg/mL)**
Nigella sativa	0.9 ±0.02
Zingiber officinale	0.33 ± 0.041
Ajwa seeds	0.05 ± 0.03

It can be easily observed that phenol and flavanoid contents and antioxidant activities of extracts of black cumin seeds (0.2 %) are double the activities of ginger and ajwa seeds' extracts separately or in combination which clearly indicates the importance of black cumin addition to the herbal tea powder.

3.3. Comparison of herbal tea powder before and after addition of *Nigella sativa* seeds

Phenolic and flavanoid contents along with the antioxidant activities and nutritive values of herbal tea powder before and after addition of black cumin seeds are illustrated in table 5.

Table 5. Comparison of herbal tea before and after black cumin addition

Analysis	Herbal tea (Ginger + Ajwa seeds) Without black cumin seed extract	Herbal tea (Ginger + Ajwa seeds) With 0.2 % black cumin seed extract
Phenol content	143.66 ± 2.02 mg GAE/g	280.22 ± 1.03 mg GAE/g
Flavanoid content	1.17 ± 0.10 mg QE/g	3.9 ± 0.35 mg QE/g
Antioxidant Activity	5.73 ± 0.51 mg/mL	12 ± 1.12 mg/mL
Nutritive value	325.96 K Cal/ 100 g	410.11 K Cal/ 100 g

It can be observed that herbal tea with black cumin seeds shows almost twofold increase in antioxidant activity along with phenol and flavanoid contents that establishes the significance of its addition to the common herbal tea mixtures. The nutritive value of tea powder has also risen significantly on addition of black cumin seeds. Touted as a 'seed of blessing', Kalonji seeds or black cumin seeds are used comprehensively in ancient medicine and various cuisines for their medicinal benefits and distinct flavour alike. These seeds form a fundamental part of ayurvedic medicine and are generally recommended for treating disorders that are related to stomach, eyes, heart and neurological conditions. These seeds are a source of power of crude fiber, amino acids, saponin, iron, sodium, calcium and potassium besides fatty acids like linolenic and oleic, volatile oils. These black seeds contain Nigella and Melatin, two components prized for highly nutritive and medicinal properties. A well-off supply of polyunsaturated fatty acids, black seeds boost metabolism, keep cholesterol under check, balance levels of insulin, facilitate blood circulation throughout the body and aid liver function. Ayurveda recommends daily consumption of 20 grams of these seeds to enhance lubrication between joints, for maintaining a healthy heart, fight breathing problems and to prevent cancer. Kalonji seeds are a depot of protein, carbohydrate, dietary fiber and fat. The seeds are also plentiful in vital minerals including calcium, phosphorus, iron, sodium and potassium and essential vitamins A, C, E and K conferring with the improved immune system, healthy liver and maintaining heart health. Cooked Kalonji seeds keep you satiated for a long time, prevent hunger pangs provide necessary fibre to the body. Like any other ingredient, Kalonji or black seeds should be taken in moderate amounts to avoid adverse effects. Studies reveal that black cumin seeds may slowdown the process of blood clotting and can worsen bleeding disorders. These seeds may sometimes bring down blood sugars very low causing hypoglycemia (Khan *et al.*, 2018, Al Jaouni *et al.*, 2019).

IV. CONCLUSION

Ethno-botanical and conventional uses of natural compounds, especially those of plant origin, have gained a great deal of interest in recent years, as they are well studied for their efficacy and widely considered to be safe for human use. The tea powder extract was quantified for proximate composition analysis of moisture content, ash content, crude fiber and crude fat that are found to be essential to maintain good health. The quantitative estimation of the crude fiber suggested that it can be a diet source which provides good digestion and palatability. Moisture and ash content estimation gave a feedback about digestibility. The estimated iron compounds can act as a base for the future work on their underlying role in deficiency disorders. Clearly, current scientific lines such as phytochemical investigations, biochemical analyses and antioxidant properties warrant attention towards *N.sativa* seeds too. The present study implies that *N. sativa* seeds have potential medicinal value.

N. sativa can be used as an adjuvant therapy along with repurposed predictable drugs to manage the patients with COVID-19. Adjuvant therapy of *N. sativa* may reduce the adverse effects of conventional medicines by helping to decrease their doses. However, more randomized controlled trials are necessitated to confirm the potential beneficial effects of *N. sativa* to treat the patients with COVID-19, as an substitute herbal medicine.

Future perspectives should be diverted to the assays by which *N. sativa* seeds prove their medicinal benefits. Based on these findings, it is also recommended to use black cumin powder in herbal tea preparation since it possessed high antioxidant activity which is beneficial for human health. With the increased understanding of its mechanism of bioactivity, the incorporation of this medicinal herb into tea powders will in-turn lead to the intake of natural antioxidants on a daily basis.

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CONFLICT OF INTEREST

The authors whose names are listed certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non financial interest in the subject matter or materials discussed in this manuscript.

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