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Comparative Analytical Study of Phytochemicals in Selected Antidiabetic Medicinal Plant Seeds in Sri Lanka

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ABSTRACT

Medicinal plants are universally used in the management of various diseases in different medical practices. A varied diversity of compounds extracted from plants may show anticancer, antibacterial, and antidiabetic activities. Comparative laboratory studies and scientific approach of efficacy related to phytochemicals found in medicinal plants in Sri Lanka have not been reported yet. This study aims to evaluate the phytochemicals in selected four different plant seeds such as *Syzygium cumini* (L.) Skeels, *Brassica alba* (L.) Rabenh, *Trigonella foenum-graecum* L. and *Nigella sativa* L., which are used in the management of diabetes mellitus in Sri Lanka. The ethanol extracts of plant seeds were subjected to the qualitative and quantitative analysis of the phytochemicals using recommended laboratory techniques. Data were analyzed by Analysis of Variance using a Statistical Analysis System (SAS) statistical package. The qualitative analysis showed that flavonoids, tannins, phenols, alkaloids, and saponins were present in all medicinal seed extracts. Based on quantitative analysis, flavonoids were found in all seed extracts, and higher amount was found in *S. cumini* (527.77 µg QE/g) followed by *T. foenum-graecum* (194.66 µg QE/g). *S. cumini* seeds contain higher phenolics (416.01 µg GAE/g), alkaloids (81.07 mg/g), and tannins (34.04 µg TAE/g) contents than other seed extracts. This study revealed that all these medicinal seeds, especially *S. cumini,* has potential as prepared standard functional products in the traditional system of medicine.

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INTRODUCTION

Diabetes mellitus is a common metabolic condition that affects the human population all over the world. About 463 million adults (20-79 years) were living with diabetes (2019) and by 2045, this will rise to 700 milion (IDF Diabetes Atlas Ninth edition, 2019). Diabetes caused 4.2 million deaths, and it is seen that the majority of the population is mainly affected by type-II diabetes, which is commonly known as non-insulin-dependent diabetes. The oxidative stress in the development and progression of diabetes is caused by the increase of free radical production and impaired antioxidant defenses. In Sri Lanka, many herbs are useful for the management of diabetes. For centuries, medicinal plants have been vastly valued worldwide as a rich source of therapeutic agents for the prevention of different ailments due to certain advantages such as they are readily available, low side effects, etc. (Pandya et al., 2011). More than 1200 species of plants have been used ethno-pharmacologically to treat diabetes mellitus (Delaviz et al., 2017).

Secondary metabolites are chemically and taxonomically very diverse compounds with incomprehensible functions. The preliminary phytochemical analysis is also included under the simple pharmacognostic techniques used in the standardization of plant material (Yadav et al., 2011). Secondary metabolites from plants such as alkaloids, flavonoids, glycosides, and phenols produce therapeutic effects in traditional medical practices. The composition of the secondary metabolites diverges from plant species to species based on the nutrient composition of the soil, climate, plant development stage, storage and the types of handling methods, etc. (Arawwawala, 2006). Although there are several medicinal plants mentioned in traditional texts for diabetes mellitus, the researchers have selected seeds of four medicinal plants such as *Syzygium cumini* (L.) Skeels, *Brassica alba* (L.) Rabenh, *Trigonella foenum-graecum* L., and *Nigella sativa* L., which are used to treat diabetes mellitus in Sri Lanka.

S. cumini (Family Myrtaceae) is entitled Java Plum (in English) and Jambul. It is extensively distributed in the forest found in India, Sri Lanka, Malaysia, and Bangladesh. All portions as fruit pulp, seed with seed coat and kernel, alone kernel, bark, and leaves are used in many health circumstances (Sharma et al., 2019). Numerous studies found that this plant has a potent antidiabetic effect (Deb et al., 2013; Tripathi & Kohli, 2014; Baldissera et al., 2016; Vihan & Brashier, 2017).

T. foenum-graecum is widely distributed throughout the world and belongs to the family Fabaceae. This plant is used medicinally as a source of many effective and powerful drugs. Out of many therapeutic activities known in *Trigonella*, its hypoglycemic effect has been reported (Sharma et al., 1990; Bordia et al., 1997; Hannan et al., 2007; Mahdavi et al., 2008; Moradi kor & Moradi, 2013).

N. sativa is a plant species that belongs to the Ranunculaceae family. This plant has different names, such as seeds of blessing and black cumin. It was described as a miraculous plant because of its unique chemical composition (Ahmad et al., 2013). Different parts of the plant have been reported as therapeutic agents in the traditional system of medicine. Seeds yield an essential oil that significantly lowers blood glucose concentration in diabetic rats (Ikram & Hussain 2014; Akhtar et al., 2020; Sadiq et al., 2021).

B. alba is the most important genus of the Brassicaceae family. Brassica serves as antioxidants and contains high levels of phenolic compounds, minerals, vitamins C, E, and carotenoids that protect against various degenerative diseases. It also has effective antidiabetic potentials (Sayeed et al., 2015).

The phytochemical compositions of the medicinal plants mostly depend on the region where those are cultivated and on the climate of that certain region. Therefore, the current study is subjected to comparatively assess the secondary metabolites present in ethanol extracts of selected above four antidiabetic medicinal plants seeds.

METHODS

Collection of Plant Materials

The selected medicinal plant seeds were collected from September to October 2020 in the Jaffna District. The seeds were botanically authenticated in the National Herbarium Centre, Department of National Botanic Gardens, Peradeniya, Sri Lanka.

Preparation of Seed Materials

The collected fresh seeds were washed with tap water and air-dried systematically at room temperature for three weeks to avoid direct loss of phytoconstituents from sunlight. The shade dried plant seeds were ground using the pulverizer and sieved up to 80 meshes. It was then homogenized to a fine powder and kept separately in air-tight containers for further analysis.

Preparation of Ethanol Extraction

The seed powder of each medicinal plant was extracted with ethanol using the cold extraction technique. A total of 50 g of powdered materials of each seed were separately weighed and placed in 500 ml of culture bottles. After that, 150 ml of 100% absolute ethanol (1:3) was added and mixed well. The lid of each bottle was covered with parafilm. The solution was kept for five days with occasional shaking by using a shaker at 150 rpm for 15 minutes every morning and evening. After that, the solution was filtered through Whatman No.1 filter paper. The part of filtered content was concentrated using a rotatory evaporator (Buchi), and the other part was kept in the refrigerator at 4°C for further uses.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanol extracts from each plant seed powder was carried out using recommended laboratory procedures. This phytochemical screening was aimed to detect the presence of phytochemicals, such as alkaloids, flavonoids, tannins, steroid glycosides, phenols, terpenoids, saponins, coumarins, reducing sugars, protein, anthraquinones and quinines (Kokate et al., 1995; Harborne, 1998; Tiwari et al., 2011; Saxena et al., 2012; Kamal, 2014; Jaleel et al., 2019).

Phytochemical screening for flavonoids (Alkaline reagent test)

Every 2 ml of filtered sample was mixed with a few drops of 20% NaOH. The formation of intense yellow color was detected. Then, a few drops of 70% diluted hydrochloric acid were added, and the yellow color was disappeared. The formation and disappearance of yellow color indicate the presence of flavonoids (Kamal, 2014).

Phytochemical screening for phenols (Ferric chloride test)

Every 2 ml of filtered sample was mixed with 2 ml of 5% aqueous $FeCl_3$. The formation of the blue color points out the occurrence of phenols (Tiwari et al., 2011).

Phytochemical screening for tannins (Ferric chloride test)

Every 2 ml of filtered sample was added to 10% of alcoholic $FeCl_3$. The formation of the black/brownish blue represents the occurrence of tannins (Harborne, 1998).

Phytochemical screening for alkaloids (*Dragendorff's Test*)

Every 2 ml of filtered sample was dissolved individually in dilute Hydrochloric acid and filtered. The filtrate was treated with Dragendorff's reagent (potassium bismuth iodide solution). The formation of a red precipitate indicates the presence of alkaloids (Saxena et al., 2012).

Phytochemical Screening for Terpenoids (Chloroform test)

Every 2 ml of filtered sample was added into 0.5 ml

chloroform, 0.5 ml of acetic anhydride, and a few drops of concentrated sulfuric acid. The formation of reddish-brown precipitate directs the presence of terpenoids (Kamal, 2014).

Phytochemical screening for anthraquinones

Every 2 ml of filtered sample was mixed with potassium hydroxide. The blood-red color shows the presence of anthraquinones (Gupta et al., 2008).

Phytochemical screening for saponin (Foam test/ Frothing test)

Every 2 ml of the filtered sample was added into 4 ml of distilled water. The solution was mixed well and shaken vigorously. If the foam is produced for ten minutes, it designates the presence of saponins (Kamal, 2014).

Phytochemical screening for quinones

Every 1 ml of the filtered sample was mixed with 1 ml of sodium hydroxide. The formation of blue, green, or red color shows the presence of quinones (Kokate et al., 1995).

Phytochemical screening for coumarins

Every 1 ml of 1% filtered sample was mixed with 3 - 4 drops of 1% KOH in absolute ethanol. The formation of yellow color directs the occurrence of Coumarins (Farnsworth, 1996).

Phytochemical screening for glycosides (Keller-Kiliani Test)

Every 2 ml of filtered sample was mixed with 0.5 ml glacial acetic acid, 3 drops of 1% aqueous $FeCl_3$ solution, and 0.5 ml H_2SO_4 concentrated. A brown ring formed between the layers indicates the entity of cardiac steroidal glycosides (Gul et al., 2017).

Quantitative Analysis of Phytochemicals

Quantitative analysis for phenols (Folin-Ciocalteu colorimetric method)

About 20 µl of each filtered sample was added to the test tube by using a micropipette. Water (1.58 µl) was added to each of above test tubes. Folin reagent (100 µl) was added to each test tubes. They were mixed well by using a magnetic stirrer, and they were allowed for 8 minutes after stirrer. Sodium carbonate solution (300 µl) was added to each stirred solution. They were heated in a water bath at 40°C for 30 minutes and permitted to cool. They were again stirred well. The absorption of each sample was measured by spectrophotometer at 765 nm wavelengths. A curve chart for each solution was prepared using absorbance and concentration. The three replicates were prepared for each sample (Singleton et al., 1999). Total phenol content was expressed as µg gallic acid equivalents per gram of extract (µg GAE/g).

Quantitative analysis for flavonoids (The Aluminium colorimetric method)

Every 0.25 ml of filtered sample was added into 4.5 ml of distilled water. An amount of 0.3 ml of $NaNO_2$ solution (5%) was added and allowed for 5 minutes. After that, 0.3 ml of 10% $AlCl_3$ was mixed and incubated for 5 minutes. An amount of 2 ml of 1 N NaOH was added, and its entire volume was made to 10 ml with distilled water and mixed well. The absorbance of each sample was measured at 510 nm by using a spectrophotometer. Blank was prepared using the above reagents and distilled water instead of the sample. A curve chart for each solution was prepared by using absorbance and concentration. The three replicates were prepared for each sample (Badarinath et al., 2010). Total flavonoid content was expressed as µg quercetin equivalents per gram of extract (µg QE/g).

Quantitative analysis for tannin (Folin-Ciocalteu colorimetric method)

Every 0.5 ml of the filtered sample was added into 3.75 ml of distilled water and 0.25 ml of folin reagent, 0.5 ml of 35% of sodium carbonate. The absorbance of each sample was measured at 725 nm using a spectrophotometer. The blank was prepared by using the above reagents with distilled water instead of the sample. A curve chart for each solution was prepared by using absorbance and concentration. The three replicates were prepared for each sample (Kavitha Chandran & Indira, 2016). Total tannin content was expressed as µg tannic acid equivalents per gram of extract (µg TAE/g).

Quantitative analysis for alkaloids

About 5 g of the three samples of each plant material were loaded into a 250 ml beaker. An amount of 200 ml of 20% of acetic acid was added and enclosed to stand for 4 hours. They were filtered, and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to each extract until the precipitous was completed. The whole solution was permitted to settle down, and the precipitate was collected by filtration through the accurately weighed filter paper. The filtrate is the alkaloid that was dried out in the oven for 4 hours and weighed. Total alkaloid content was measured as mg per g of air-dried material (Edeoga et al., 2005; Aliyu et al., 2008).

Statistical Analysis

Data were statistically scrutinized by Analysis of Variance (ANOVA) using a SAS statistical package (version 9.1.3), and mean separation was completed by Least Significance Difference (LSD). Statistical analysis was done for three replicates of each medicinal plant seeds.

Table 1. Qualitative phytochemical screening of the selected medicinal plant seeds

Phytochemicals	*Syzygium cumini*	*Brassica alba*	*Trigonella foenum-graecum*	*Nigella sativa*
Flavonoids	*	*	*	*
Tannins	*	*	*	*
Phenols	*	*	*	*
Glycosides	*	-	-	-
Terpenoids	*	-	-	*
Alkaloids	*	*	*	*
Anthraquinones	*	-	-	*
Saponins	*	*	*	*
Quinones	*	-	-	*
Coumarins	*	*	*	-

(*): Presence of constituents, (-): Absence of constituents

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The Preliminary phytochemical screening for several functional groups was presented in Table 1. The results of phytochemicals screenings showed that the ethanol extract of selected medicinal plants seeds contained flavonoids, tannins, phenols, alkaloids, and saponins (Figure 2 & 3). Glycosides are only represented in *S. cumini*. Terpenoids, anthraquinones, quinones are absent in *B. alba* and *T. foenum-graecum*. Coumarins are absent in *N. sativa*. Phytochemicals such as saponins, anthraquinones, glycosides, phenols, tannins, alkaloids, flavonoids are known as the hypoglycemic properties (Mujeeb et al., 2014). Saponin showed multifunctional activities in the human body, such as antifungal activity, reducing blood cholesterol level and risk of cancer, and stimulating the immune system (Moses et al., 2014). *S. cumini* has been known as a multifunctional medicinal herb and has been used in traditional medicine systems as a starting material to make antidiabetic drugs for treating diabetes mellitus. Seeds of *S. cumini* are also used as astringent and diuretic, hypoglycemic, antipyretic, anti-inflammatory, psychopharmacological, hypolipidemic, and antioxidant agents (Sharma et al., 2019).

The phytochemical screening results of *S. cumini* in the present study comparable with the previous studies carried out in different countries (Murti et al., 2012; Kamal, 2014; Prabakaran & Shanmugavel 2017; Aziz & Banerjee, 2018).

Quantitative analysis of Phytochemicals

The Quantitative phytochemical analysis of different medicinal seeds is tabulated in Table 2. All of the total content of phytochemicals was obtained using a calibration curve drawn for each of four phytochemicals (Figure 1). The comparison of the phytochemical constituents of the plant seed extract of *S. cumini, B. alba, T. foenum-graecum, N. sativa* showed that all the four different types of phytochemical constituents are highly present in seeds extracts of *S. cumini* rather than other medicinal plant seeds. GC-MS analysis of black plum seed extract reveals that the seed contained ten different compounds which possess antimicrobial, anti-inflammatory, antioxidant activities (Jaleel et al., 2019).

As apparent Table 2, the alkaloid contents were highly found 81.07 mg/g in seeds extract of *S. cumini* compared with the seeds extracts of *N. sativa* (22.12 mg/g). Alkaloids are nitrogenous organic molecules and naturally occurring (Kurek, 2019). The major use of alkaloids in human body is the management of diabetes. Alkaloids do the reverse conversion of starch into sugar in blood levels in the human body. Alkaloids exert a wide range of antidiabetic activities through different mechanisms (Gaikwad et al., 2014).

The seed extract of the *S. cumini* has higher phenol content (416.01 µg GAE/g) than the *T. foenum-graecum* seed extract (11.85 µg GAE/g). Phenolic compounds are powerful natural antioxidants and interact with protein and inhibit amylase enzymatic activity. Therefore, it can be controlling the glycemic index of food products and is involved in the free radical defense mechanism in human cells (Shahidi & Ambigaipalan, 2015; Klim et al., 2016).

The flavonoid content was highly found (527.77 µg QE/g and 194.66 µg QE/g) in the *S. cumini* and *T. foenum-graecum* seed extracts, respectively. Flavonoids are polyphenolic compounds that also have antidiabetic potential. It is also involved in the free radical defense mechanism in human cells, resulting in reducing excessive oxidative stress generated in human cells

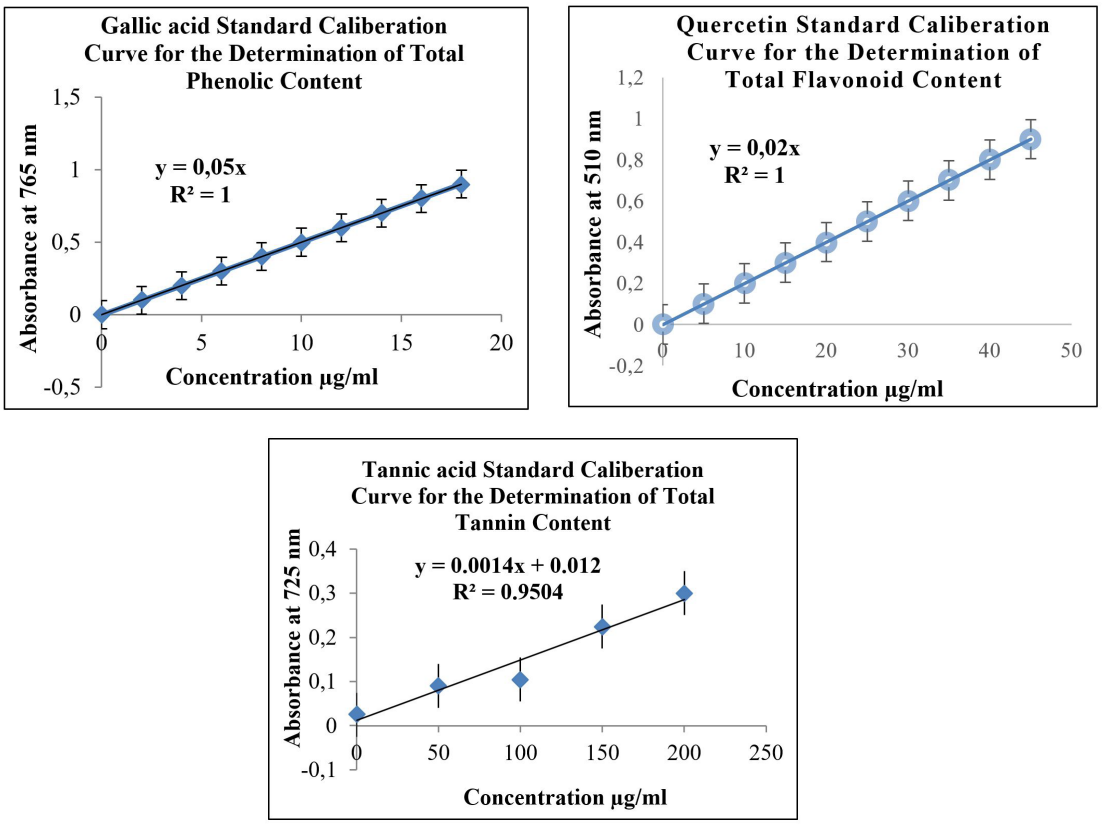

Figure 1. Standard curves for quantitative analysis

Table 2. Quantitative analysis of phytochemicals of seeds extracts of selected medicinal plants

Plant Materials	Phenols (µg GAE/g)	Tannins (µg TAE/g)	Flavonoids (µg QE/g)	Alkaloids (mg/g)
Syzygium cumini	416.01±10.88	34.04±3.50	527.77±14.03	81.07±3.04
Trigonella foenum-graecum	11.85±1.41	24.94±2.35	194.66±5.22	31.30±1.43
Brassica alba	45.79±2.78	33.04±1.23	42.97±1.20	33.39±2.34
Nigella sativa	25.52±1.24	26.96±2.31	24.33±0.84	22.12±1.43

Results are expressed as Mean ± SD and statistical significance was evaluated by one-way analysis of variance (ANOVA).

under unfavorable conditions (Tungmunnithum, 2018; Al-Ishaq et al., 2019).

The tannin content was found to be 34.04 µg TAE/g, 33.04 µg TAE/g, 24.94 µg TAE/g, and 26.96 µg TAE/g in seed extract of the *S. cumini*, *B. alba*, T. *foenum-graecum*, and *N. sativa*, respectively. Tannins are considered anti-nutrients. However, some tannins have shown medicinal properties such as anti-carcinogenic activity, antimicrobial activities, etc. (Chung et al., 1998).

Previous study reported that the *S. cumini* contains jambosine, gallic acid, ellagic acid, corilagin, glycoside jambolin or antimellin, resin, albumin, chlorophyll, flavonoids, related tannin, 3, 6-hexahydroxydiphenoylglucose and its isomer 4, 6-hexahydroxydiphenoylglucose, 1-galloylglucose, 3-galloylglucose, quercetin (Omar et al., 2012). Its seeds have higher alkaloid content than other plant parts (Sing et al., 2015).

Other study reported constituents of the *S. cumini* seeds tannin to 19%; gallic acid, 1% to 2%, and fatty acids (palmitic, stearic, oleic, and linoleic) (Gowri & Vasantha, 2010). *N. sativa* seeds contain two different types of alkaloids; that is isoquinoline alkaloids e.g., nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine and also contain alpha-hederin, a water

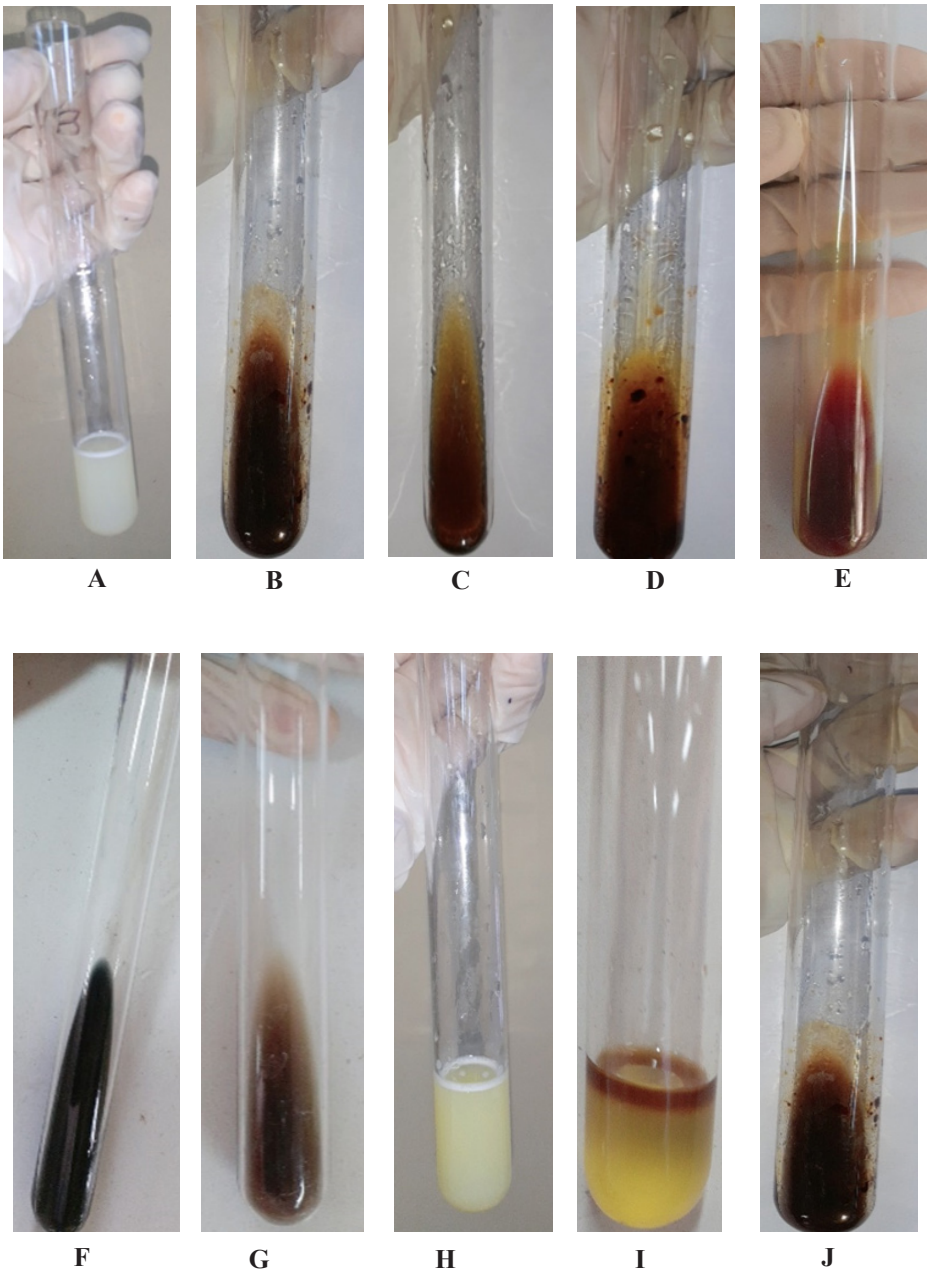

Figure 2. Observations of qualitative phytochemical analysis of *S. cumini* seeds (Saponin (A), Coumarin (B), Terpenoid (C), Anthraquinone (D), Alkaloid (E), Phenol (F), Tannin(G), Flavonoid (H), Glycoside (I), Quinone (J))

soluble pentacyclic triterpene and saponin, a potential anticancer agent (Al-Jassir,1992; Atta-Ur, 1995). Seven phenolic acids and flavonoids were determined in four different fractions of the *N. sativa* seed extract, and the majority of phenolic acids are ester-bounded to cell walls, followed by the corresponding esters and glycosides (Robbins, 2003). Quantitatively, tannin content was highest (7.75±0.06%) in the seeds of mustard (Farquar, 1996), and its phenolics and flavonoids are considered as critical active components (Radford et al., 1986). Further, thin layer chromatography analysis identified six different phenolic compounds in all the genotypes of mustard seeds (Sharma et al., 2017). Previous research found that the water extract of fenugreek seeds has alkaloids, flavonoids, phenolic compound, and tannins (Yadav & Chowdhury, 2017). A study indicates that crude fenugreek seeds could be considered a rich source of bioactive phenolic compounds. HPLC coupled to both ESI/MS and DAD was used to separate and identify 24 flavonoid glycosides in the extract of crude fenugreek seeds (Benayad et al., 2014; Keskes et al., 2018).

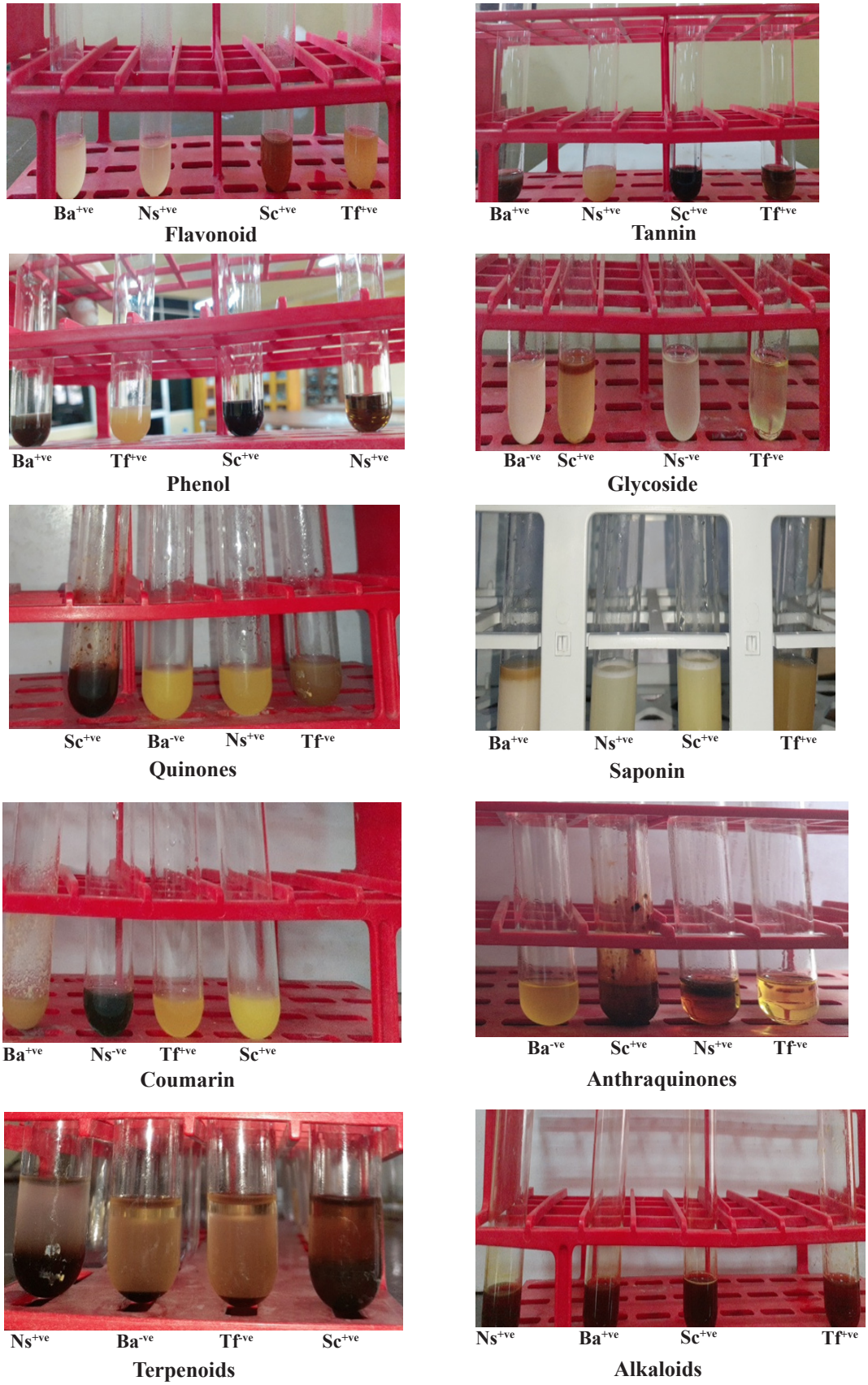

Figure 3. Observations of qualitative phytochemical screening of the selected medicinal plant seeds (Ba: *B. alba*; Ns: *N. sativa*; Sc: *S. cumini*; Tf: *T. foenum-graecum*; -ve: negative; +ve: positive)

Phytochemical analysis of the ethanol seed extracts from the selected medicinal plants demonstrated the occurrence of phenols, flavonoids, alkaloids, tannins, saponins as the major phytochemicals. These constituents are considered as the main bioactive constituents to control diabetes mellitus in the human body. Each constituent is present in a high concentration level by *S. cumini* than other medicinal plants seed extracts. Based on the experimental and statistical data, *S. cumini* can be introduced as starting material to produce pharmaceutically valuable drugs for treating diabetes mellitus.

CONCLUSION

This study revealed that ethanol extracts of all medicinal seeds contain essential phytochemicals. These medicinal seeds can be used as monotherapy or add-on therapy in diabetes management. A seed of *S. cumini* is rich in important secondary metabolites and contains favorable proximate composition with a higher total phenolic and flavonoid content than other seed extracts. All these medicinal seeds, especially *S. cumini* can be practiced as prepared standard functional products in the traditional system of medicine. The current study results will deliver data supporting future scientific-related clinical trials in diabetic subjects.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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