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Comparative Study of Two Turkish Coffee Brands Sold in Saudi Market

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ABSTRACT

Objective: Globally, coffee is considered one of the most widely consumed morning beverages due to its intense taste and stimulating effects, which are mostly ascribed to its caffeine concentration. The purpose of this study is to quantitatively assess the amount of caffeine present in two commercially available coffee brands that were bought from the local markets in Saudi Arabia, Al-Kharj Province and were originally manufactured in Turkey. Methods and materials: UV-Visible spectrophotometer (UV-Vis), a dependable and precise technique for quantifying caffeine that was used to determine the amount of caffeine present in each sample. The study also examined the presence of other bioactive substances, such as catechins, theaflavins, and tannins, in addition to caffeine, by qualitative analysis. By comparing the caffeine content of the two brands, this study aims to offer insightful information about the market's variability in caffeine levels, which can help consumers understand the possible health effects of coffee consumption, especially with regard to its stimulating properties and the possibility of caffeine-related side effects. Results: The Saudi Food and Drug Authority instructions now mandate listing the percentage of caffeine in milligrams per 100 millilitres or per cup on any cafe menus. This study provides insights into the caffeine variability in commercially available coffee brands, aiding consumer awareness of caffeine intake, emphasizing that there may be differences in

coffee bean type, roasting process, and caffeine extraction efficiency. This research provides valuable insights for consumers regarding caffeine intake and its potential health implications in the future.

Keywords: Turkish coffee, UV spectrophotometer, caffeine, phytochemical screening.

1-Introduction

There is now a lot of research being done on the effects of certain biochemical components found in coffee beans on biological systems. Coffee is one of the most widely consumed beverages worldwide, valued for its stimulating, antioxidant, and metabolic benefits, with daily consuming about 2.3 billion cups all-over the world [1] and adults are consuming 3-4 cups/d providing 300-400 mg/d of caffeine as a moderate amount [2]. It belongs to Rubiaceae family, with the two most prominent species; *Coffea arabica* and *Coffea canephora* (Robusta). These species differ in -flavor profile, caffeine content, and chemical composition [3].

Hydroxycinnamic acids, chlorogenic acid and its derivatives, are compounds found in coffee beans that can help avoid several chronic degenerative diseases [4, 5]. Coffee both green and roasted is a complicated chemical mixture that contains high levels of caffeine and chlorogenic acid, diterpenes called cafestol and kahweol, which have been linked to coffee's ability to raise cholesterol, are abundant in unfiltered coffee [2]. Additionally, because these compounds contribute to the flavor and aroma of coffee beverages, they play a crucial role in the quality of coffee beans and may be help to improve health in case of liver disorders and prevent many aliments such as Parkinson's disease and diabetes mellitus (type 2) [2, 4, 5].

Throughout history, coffee has been appreciated for its energizing properties. Modern research has further highlighted its potential health benefits, including boosting cognitive function and enhancing alertness due to its caffeine content. It provides antioxidant protection through chlorogenic acids [2, 3, 6]. In addition, supporting metabolism, influencing fat oxidation and insulin sensitivity [7].

Otherwise, the biological and pharmacological effects of coffee were observed as CNS stimulation, enhances alertness, cognitive function, and reduces neurodegenerative risks, antioxidant, reducing oxidative stress, and inflammation due to it rich in polyphenols [3], boosts fat oxidation, supports weight control [8], and improves insulin sensitivity [7], lower stroke and heart disease risk; affects cholesterol levels [9], reduces the risk of cirrhosis and liver cancer, supports liver function [10], and helps in aids digestion but may increase acid reflux risk [11].

The effect of caffeine on humans are varying according to its concentration. Several physiological and psychological effects, including central nervous system stimulation, bronchial muscle relaxation, gastric acid production, and diuresis, are brought on by consuming high concentrations of this substance. Increases in caffeine concentration *in vivo* are also a major indicator of a number of illnesses, such as asthma, kidney disease, and heart disease [4, 12].

Additionally, caffeine alters our sleep patterns, performance, and focus [12, 13]. The gastrointestinal tract tends to absorb caffeine quickly and completely, and it is then distributed throughout the body [14]. However, it is not eliminated from circulation until it is metabolized, first into paraxanthine, theobromine, and theophylline, and then into a derivative of uric acid and diaminourcil, which is then eliminated from circulation [4]. The amount of time needed for caffeine level to drop by 50% due to biotransformation and excretion is therefore 5 – 6 hours [14]. Effects such as cardiovascular effects, respiratory stimulation, diuresis, mild anxiety, and an increase in stomach secretion would be noted when the peak plasma level of caffeine concentration was between 15 and 30 M. but an acute poisoning symptom could show up when its levels become 150 – 200 M. These include extreme anxiety, agitation, tense muscles, twitches, and heart problems including tachycardia [15].

In 2005, caffeine was considered a substance of abuse by the International Olympic Committee if its concentration in human urine exceeds 12 μg/mL [16]. However, in addition to the physiological and psychological impacts of caffeine, coffee quality has also been assessed using chemical tests of caffeine in coffee samples. Higher caffeine levels have been linked to lower-quality samples as compared to other Arabic samples [17]. Coffee decaffeination is becoming more and more popular as a result of the above listed negative consequences. In the coffee industry, decaffeination accounts for around 10% of global green coffee production and 20% of coffee imports to certain European nations [4, 15].

Coffee decaffeination by chemicals in the business is costly, alters the coffee's flavour and aroma, and lowers the quality of the coffee cup. Recently, researchers have been looking for coffee beans that are naturally decaffeinated in light of the issues with the decaffeination procedure. As a result, three naturally decaffeinated types of Ethiopian coffee trees were identified by Brazilian researchers after screening 300 of them; they called these varieties AC1, AC2, and AC3. When these types were analyzed, the caffeine content was determined to be 0.07% lower than that of natural coffee beans [4, 18]. It is believed that this discovery would

offer a substitute for the currently available artificially decaffeinated coffee. To determine the provenance of decaffeinated coffee beans purchased all over the world, it is crucial to provide a straightforward technique for analyzing caffeine in coffee beans.

Our goal is to review the chemical properties, composition, and diverse uses of some biochemical compounds found in coffee brands bought from the Saudi market, such as xanthine alkaloids, as well as the various physical and chemical methods that have been developed to analyze these compounds in coffee. In addition, we tried to confirm the caffeine concentrations of the two tested brands as their manufacturers did not mention the amount outside their boxes.

2-Materials and methods

Two coffee brands were bought from Saudi market which produced by Turkish original companies (A and B) which used in this research. All chemicals, reagents and caffeine were purchased from Sigma-Aldrich Co. LLC (p.a. grade).

2.1- Phytochemicals identification in two coffee brands [19] [20].

Every chemical test was carried out and documented.

2.1.1- Identification of caffeine, theobromine and theophylline (xanthine alkaloids):

1-Murexide reagent: Hydrochloric acid (Three-drops) and potassium chlorate ($KClO_3$, few of powder) were added to caffeine crystals → set on water bath till dryness → red-purple color is formed.

2-Wagner's test: Iodine-potassium iodide reagent (iodine (1.3 g) and potassium iodide (2 g) in 100 ml of dist. water) and conc. hydrochloric acid were added to sample solution → a brown precipitate is formed.

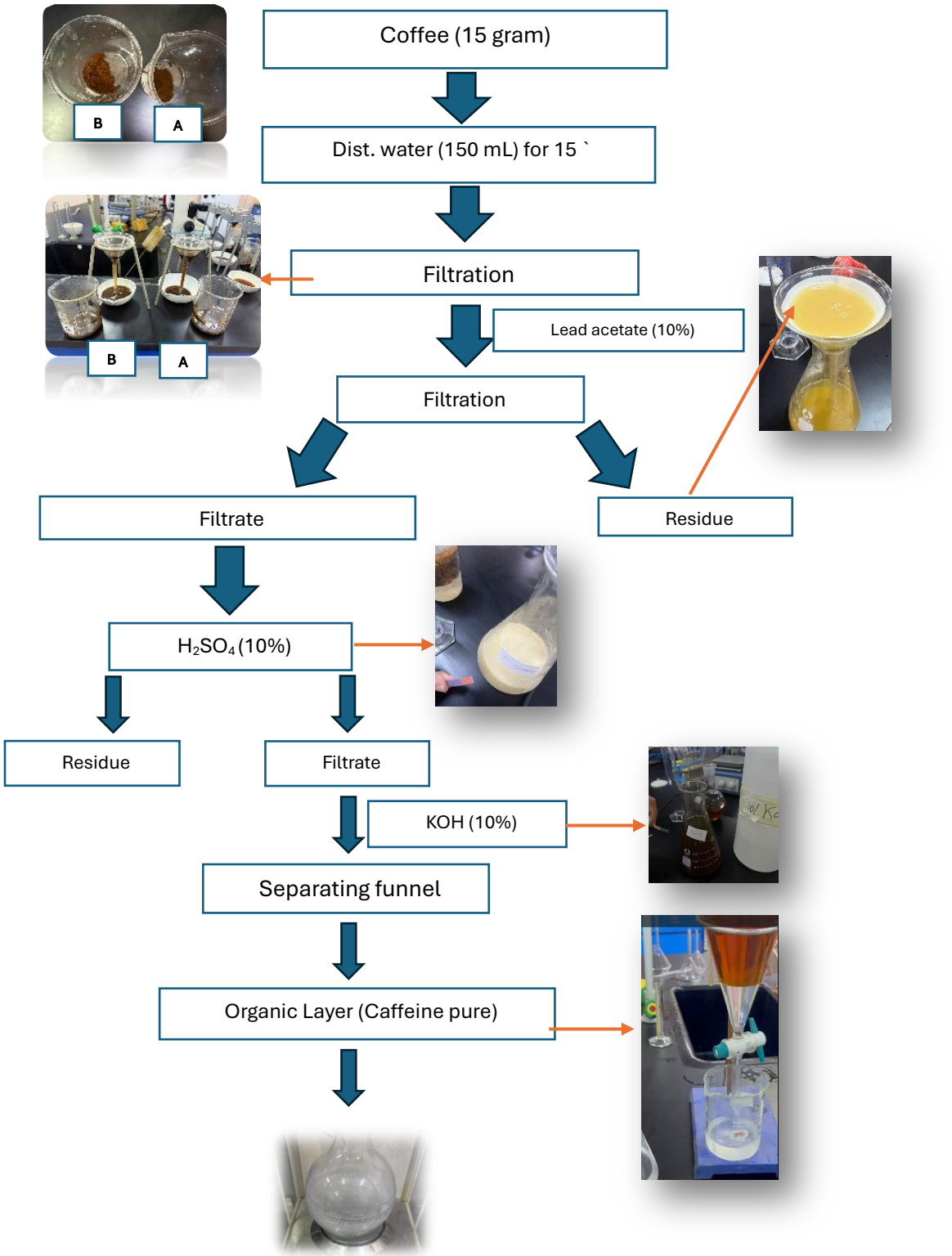
2.1.2- Identification of different substances:

3- Molisch's test: Molisch's Reagent (Two-drops) and conc. H_2SO_4 (1mL) were added to coffee solution (2mL) → A red -violet ring appears in between the two solutions (Carbohydrates).

4- Ninhydrin test: Ninhydrin reagent (few drops) was added to coffee extracts (1 mL) → Purple color (Amino acids).

5- Ferric chloride ($FeCl_3$) test: 1 % ferric chloride (1 mL) was added to coffee extract → blue, green or brownish green color (Tannins: condensed/hydrolysable).

6-Lead acetate test (10%): 10% lead acetate solution was added to 1mL of coffee extract → A bulky white precipitate (Phenolic compounds).



Flowchart 1. Pure caffeine from two coffee brands bought from Al Kharj city.

7- Froth test: 5 ml of the coffee extract was vigorous shake →A two cm layer of foam formed (Saponins).

8- Liebermann test: conc. H_2SO_4 (1mL) was added to coffee solution (1mL) → reddish ring at the junction of 2 layers (Sterols).

9- Salkowski test: conc. H_2SO_4 (1mL) and acetic anhydride (few drops) were added to coffee solution (2mL) → red color and Lower layer turns to yellow after few minutes (Triterpenes).

2.2- Thin Layer Chromatography analysis

The TLC was used to assess the purity of separated caffeine from various materials by comparing it to standards (xanthines). After being dissolved in ethanol-water (8:2, v/v), the two brands and standards were put onto precoated TLC. On the silica gel F_{254}, the chromatographic separations were carried out by using 5% Acetic acid: ethyl acetate (5:95, v/v) as a mobile phase. The caffeine spots were then obtained by spraying a reagent after the visualization was detected under a UV lamp at 254 nm and 312 nm, and R_f values were computed, as shown in Figure 2 [21-23]. A solution of 1 gram of iodine dissolved in 25 millilitres of acetone and another of 2.5 grams of ferric chloride and 5 grams of tartaric acid dissolved in 25 millilitres of dist. water were combined to create the reagent. As seen in Figure 2, solutions A and B were combined and employed as a spraying reagent to identify xanthine alkaloids [24, 25]. The solvents, standards, and reagents were obtained from Sigma-Aldrich Co. LLC (p.a. grade).

2.3- Quantitatively detection of caffeine concentration in the two coffee brands

An instrument called a Shimadzu UV-Vis Spectrometer (double beam- UV-1800 (Model TM2) was used to quantitatively analyze caffeine. The λ_{max} (200 to 400 nm) was measured by scanning the standard solution. The results revealed a single strong absorption band in an absorption spectrum at λ_{max} = 275 nm. The linear range of samples analysed was calculated using a standard linear calibration curve and the equation (y = 0.6918x + 0.0219) (Figure 3). The standard calibration curve was linear over the 10 till100 µg/ml of caffeine range, and the correlation factor had an approved value of 0.9998. The quantitative amount of caffeine in the samples (µg/mL) was then determined using the standard curve [21]. In short, a standard of caffeine was made by dissolving 1 mg of caffeine in 100 mL of dichloromethane (100 mL volumetric flask). The working standard solutions (1, 2, 4, 6, 8, and 10 mg/100 mL) were used in this study. The absorbance of each solution was measured at 275 nm. The absorbance readings were then plotted against concentrations to construct a standard calibration curve [21, 26].

2.4- Physicochemical determinations of two Turkish coffee [24, 27, 28]

Moisture content: Separately, two grams of each coffee brand were kept for two hours at 105 ^{0}C, and then they were chilled for thirty minutes in a desiccator. The moisture content of each sample (mg/g) was determined using the weight loss [27].

Extractive value: Five grams of coffee brands were combined with 100 millilitres of boiling dist. water, boiled gently for one hour, filtered, and then dried in an oven at 100 ^{0}C until completely dry (16–24 hours). The samples were then weighed, and the total crude extract and yield % were then determined [28].

Total ash: Three grams of coffee brands were individually burned in a muffin crucible at 500–600 ^{0}C for 30 minutes and then cooled in a desiccator for another 30 minutes. The weight loss was utilized to determine each sample's total content (A_1) (mg/g) [24, 28].

Acid insoluble ash: The acid insoluble ash (mg/g) was calculated by adding 25 mL of 37% hydrochloric acid to the yield (A_1), boiling it for five minutes, filtering it through ashless filter paper, drying the acid-insoluble material, and then lighting it at 500 ^{0}C to a constant weight [24, 28].

Water soluble ash (A_3): 25 mL dist. water to the total ash (A_1). After five minutes of boiling, strain the water through ashless filter paper and rinse the residue with hot dist water. Water is used to create residue (A_2), which is then ignited for 15 minutes at 450 ^{0}C. A_3 (mg/g) = A_1-A_2 [24, 28].

3- Results and discussion

The fact that coffee is one of the most widely consumed beverages worldwide and that it is a rich source of dietary antioxidants has led to the realization that coffee plays a significant role in dietary antioxidant consumption [29]. Previously, the association between coffee's phenolic profiles, browning index, and antioxidant capacity was discovered through the use of principal component analysis (PCA). The primary phenolic component found in coffee beans was chlorogenic acid. Therefore, roasting coffee at 180 °C for 20 minutes or 220 °C for 10 minutes is the most important method to preserve a good level of phenolic compounds while also maintaining a nice taste [30]. Meanwhile, coffee roasting results from the moderate boiling of the green coffee components, which releases highly active low molecular weight phenols [31], depending on the place of origin, the antioxidant content of *Coffee arabica* was 0.95, 1.01, 1.03, and 1.17 mmol Trolox/g in Brazil, Columbia, Ethiopia, and India, respectively [32].

The antioxidant activity of the coffee extracts from different varieties were significantly higher ($p<0.05$) [29]. The overall antioxidant activity increased from the green coffee beans to the light roasting degree, which is consistent with previous researches [33, 34]. The researchers explained that the genetic variety between the coffee types is the cause of these variances [29]. Additionally, previous data examined the chemical composition and antioxidant capabilities of coffee beans at various roasting stages: green coffee, filter-roasted coffee, and espresso-roasted coffee. Results demonstrate that filter-roasted coffee possesses the greatest radical scavenging activity, as indicated by its lowest IC_{50} value for 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition [35].

3.1- Concentration of caffeine in two coffee brands

Coffee roasting is regarded as a crucial procedure that affects customer choice, nutrient content, and coffee cupping quality. The flavor and composition of green coffee can be altered by complex chemical processes that occur throughout the roasting process [29]. As shown in previous studies, the roasting conditions were effected on some physicochemical characteristics of coffee beans as browning index, caffeine content, color, phenolic acids, hydroxymethylfurfural (HMF), and antioxidant capacity [30].

At the current study, caffeine amounts were found different in the two brands which manufactured in Turkish that they were to be 39.1 and 21.46 mg/g for brand A and brand B, respectively. The highest caffeine concentration was showed in brand A. The current results are completely different from the previous research by Belay et al. [36]. The results observed with low caffeine concentrations rather than the previous published data were enhancement and supporting that two brands have high quality (Figure 1).

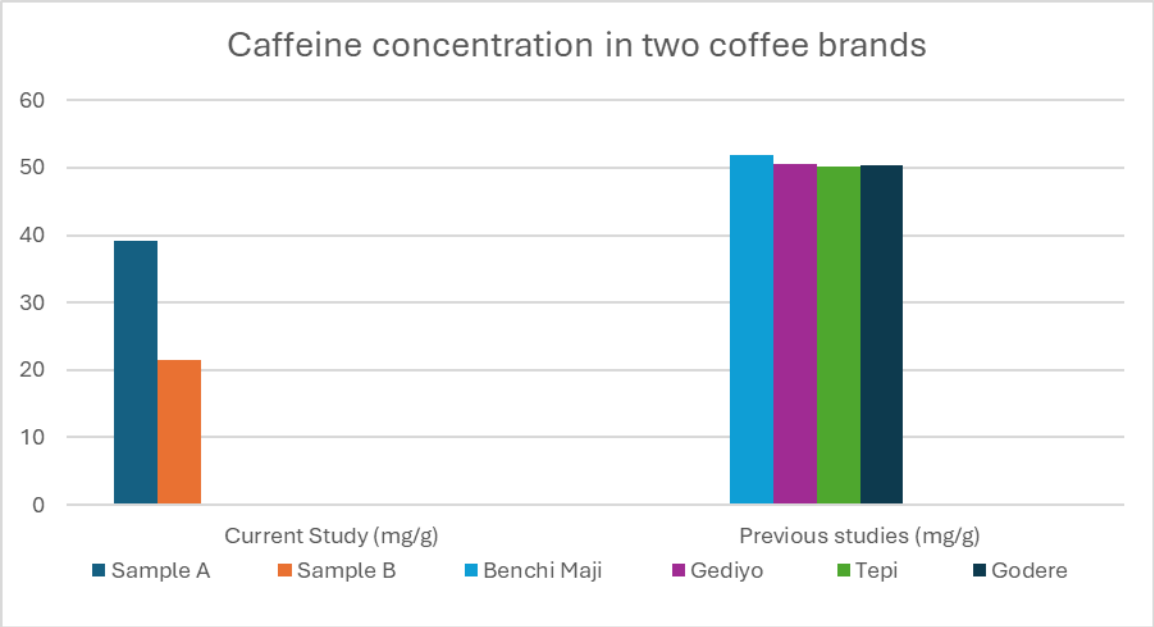


Figure 1. Caffeine contents in two Turkish coffee brands, compared with the previous research [36].

3.2- Phytochemical determination in the two coffee brands

Many phytochemicals were detected including alkaloids, phenolic compounds and tannins, from other hand, amino acids, carbohydrates, saponins, triterpenoids and steroids were absent in the two coffee brands (Table 1).

Table 1. Phytochemical identification of the two coffee brands.

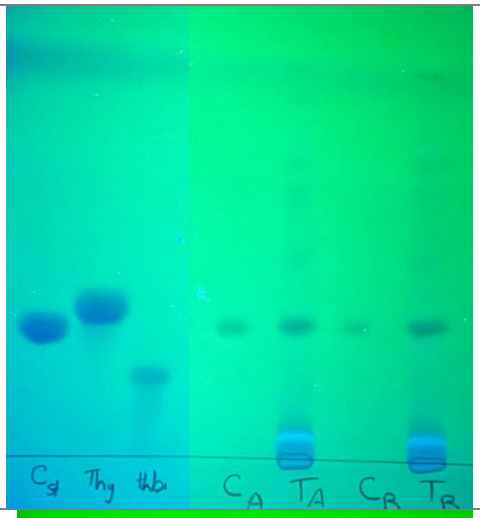
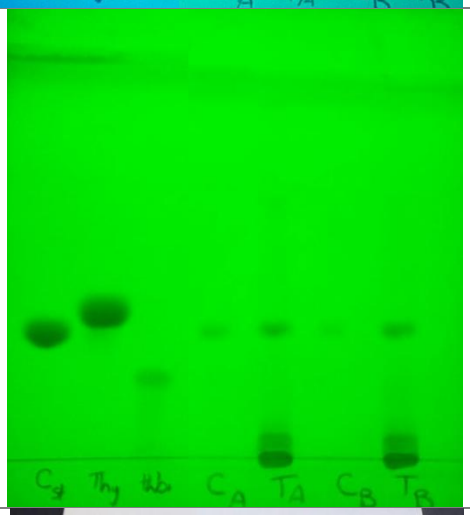
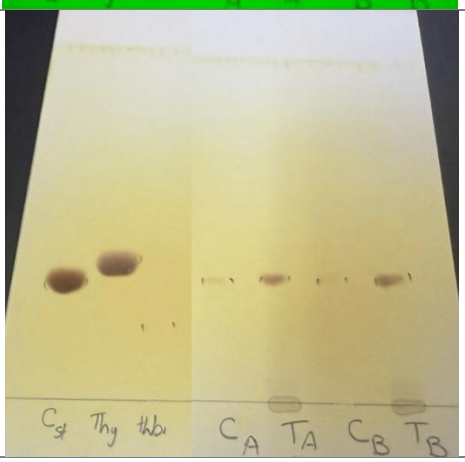
		Sample A	Sample B
	Alkaloids tests		
1	Caffeine and its derivatives	+	+
2	Caffeine and its derivatives	+	+
3	Carbohydrates	-	-
4	Amino acids	-	-
5	Tannins	+	+
6	Phenolic compounds	+	+
7	Saponins	-	-
8	Sterols	-	-
9	Triterpenes	-	-

+: present; -: absent

3.3- Thin Layer Chromatography results

The TLC profile, which was analysed by 5% acetic acid: ethyl acetate (5:95, v/v), was used to identify the total hot extracts and its caffeine pure of the two brands. In contrast to the standard of xanthine's alkaloids (caffeine, theophylline, and theobromine), caffeine spot was identified by

UV 254/312 light as a dark-blue spot with R*f* = 0.34 and then sprayed with a ferric chloride: iodine mixture to represent a dark-brown color (Figure 2).

	TLC Plate
at 254 nm	
at 312 nm	
With $FeCl_3$: I_2 (1:1)	

C_{st}: Caffeine standard; Th_y: Theophylline standard; Thb: Theobromine standard; C_A= pure caffeine of brand A; T_A= brand A total hot extract; C_B= pure caffeine of brand B; T_B= total hot extract of sample B total hot extract sprayed with $FeCl_3$: I_2 (1:1).

Figure 2. TLC plates of the two coffee brands.

3.4- Caffeine detections and its calibration curve.

However, the two coffee brands manufactured in turkey, sample A showed the higher caffeine content with 0.338 mg/mL than sample B with 0.293 mg/mL when determined by using UV-Vis spectrophotometer technique (Figure 3).

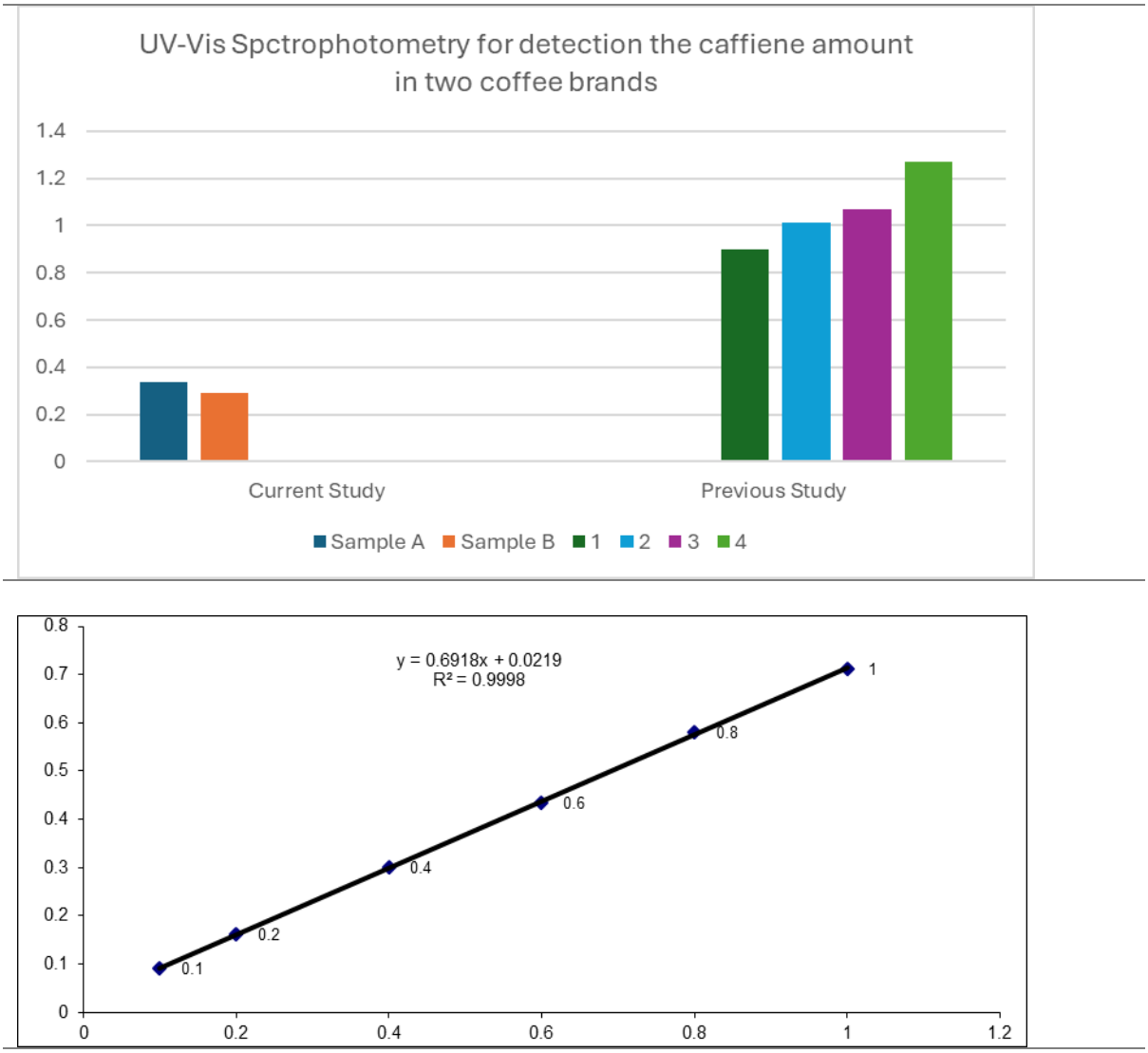


Figure 3. Caffeine concentration of the two coffee brands with its standard calibration curve by UV-Vis spectrophotometer **[37]**.

3.5- Physicochemical determinations of two coffee brands

The hot procedure to obtain the total extract had showed the highest values compared to the cold method. The extractive content of the two coffee brands from the hot procedure showed yields with 28.12 and 31.15 % of brands A and B, respectively. However, cold method showed yields with 11.88 and 14.25 % of brands A and B, respectively. As mentioned in the previous study, the extract content ranged from 25.57– 34.34 % that was completely consistent with the current

results 28.12 – 31.15 % [38]. The higher percentage of hot extractions were explanation as the more complex organic molecules in coffee are thermally broken down into simpler organic compounds, the higher the roasting level. The degree of roast directly correlates with the level of ground coffee essence. The portion of ground coffee that dissolves in water is referred to as coffee essence. A mixture of organic and inorganic chemical components, including acid, sugar, chlorogenic acid, caffeine, triglonelin, melanoidin, and minerals, make up the coffee essence found in coffee grounds. The roast level has an impact on the juice content as well. Table 2 displays the coffee extract's test findings, which are as follows. Because the amount of coffee essence varies depending on the type of coffee, these results suggest that the sample with a lower value of coffee essence has a higher level of coffee purity [38].

According to the physicochemical results, the two coffee brands showed the close results with 3.4% and 5.66% of brand A and 3.07% and 5.52 % of brand B in the total ash and the moisture contents, respectively. In previous studies, the roasted coffee showed total ash contents in the range of 4.3– 4.6% and 3.99 – 9.44 %, but our results showed no consistent with these published data [39, 40]. Significantly, the two brands showed no precise quantity of impurities which their total ash contents did not exceed 5.00% (Table 2 and Figure 4) [39].

Our results of the moisture contents of the two brands A and B with 5.66% and 5.52%, respectively, are very close with the roasting coffee, that showed moisture content in the range from 3.33– 4.9% [40]. In addition, the previous study of the arabica coffee showed the same percentage of the moisture content with our tested brands in the range from 5.8– 6.7% [41]. Moreover, at the same study the acid insoluble ash content was completely various from our current study, which showed 2.86 and 3.3% of acid insoluble ash contents of brand A and B, respectively, that validates the finding of high earthy matter content results [41].

All results considered, the physicochemical findings supported the provided information about the fluctuations in caffeine concentrations in the Saudi market (Table 2 and Figure 4).

Table 2. Caffeine amounts in the two coffee brands with its physicochemical analysis, compared with previous studies.

	Cold maceration yield	Hot decoction yield	Caffeine amount (mg/g)	Total ash (3 g)	Acid-insoluble ash (3 g)	Water soluble ash (3 g)	Moisture content (2 g)
				Values in percentage (%)			
Current study (Turkish brands)							
Sample A	11.88	28.12	39.1	3.4	3.3	2.19	5.66
Sample B	14.25	31.15	21.46	3.07	2.861	1.87	5.52
Previous studies (5, 10, or 30 g)							
Benchi Maji **[36]**			51.90				
Gediyo yergachefe **[36]**			50.60				
Tepi **[36]**			50.20				
Godere **[36]**			50.3				
Arabica Coffee **[41]**				1.7– 3.3	0.0098– 0.02		5.8– 6.7
Arabia coffee **[39]**				2.5– 4.5			
Roasted coffee **[39]**				3.99 – 9.44			
Roasting coffee **[40]**				4.3– 4.6			3.33– 4.9
Commercial coffee **[38]**		25.57– 34.34					

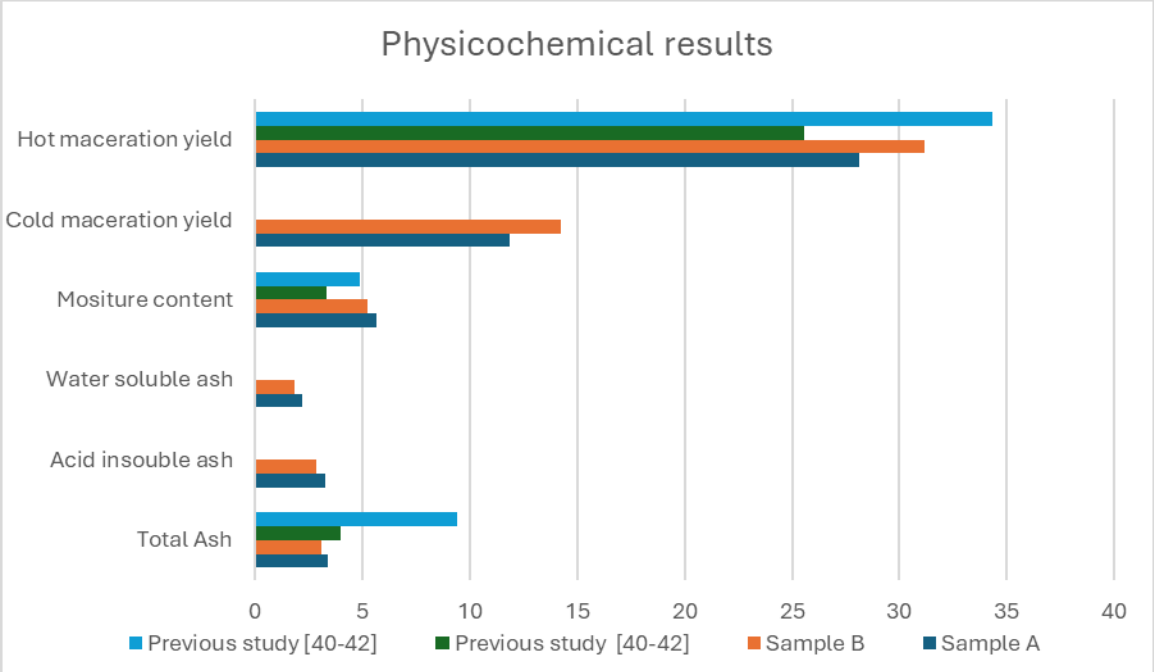


Figure 4. Physicochemical results for two coffee brands compared with previous studies.

4- Conclusion

UV/Vis spectrophotometry results were approached to determine the caffeine in two coffee brands (A and B) manufactured in Turkish, and collected from the Saudi market. They were slightly different in quantity. Compared to previous studies, the lower caffeine concentrations in these brands suggest they may be of high quality. However, further studies are needed to confirm the correlation between caffeine content and coffee quality. In addition, significantly, the two coffee brands showed no precise quantity of impurities and their total ash contents did not exceed 5.00%. The higher percentage of hot extractions ranged from 28.12 and 31.15 % explicated by breaking down the organic components into simpler organic compounds which means a higher roasting level for these brands. Finally, we hope all caffeinated products will label the quantity of their caffeine in the future all over the world. Nowadays, the Saudi Food and Drug Authority requires restaurants and cafes that serve drinks containing caffeine to indicate the amount on their menus, and the percentage presented in milligrams per 100 ml or per cup and include an explanatory statement stating that the maximum consumption limit for an adult is 400 milligrams per day.

Author Contributions: Conceptualization, project administration, and supervision, S.H.S.; methodology and formal analysis, S.H.S., M.M.A., and R.H.A.; validation, visualization, investigation and writing—original draft preparation, S.H.S.; and funding acquisition and writing—review and editing, S.H.S. and A.I.F.; resources, A.I.F. All authors have agreed to the published version of the manuscript.

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- Conflict of interest: No conflict of interest.

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