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Antidiabetic effect of black pepper, turmeric, and ajwa date pulp, seed, and their mixtures as antioxidants in alloxan diabetic rats

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ABSTRACT. Testing natural antihyperlipidemic substances is necessary due to the increasing prevalence of diabetes mellitus, which leads to chronic hyperglycaemia and poses a significant threat to public health and well-being worldwide. The present study investigated the anti-diabetic effect of black pepper, turmeric, and ajwa date pulp + seed and their mixtures as hypolipidemic, antioxidant and protective agents on the liver and kidney by examining histological changes in alloxanised diabetic rats. Eleven groups consisting of eighteen males and eighteen females were divided into a normal control (non-diabetic), a positive control (diabetic), positive groups receiving glibenclamide (10 mg kg^{-1} body weight), black pepper (BP), turmeric (T), ajwa pulp (AP), ajwa seeds (AS) aqueous extracts and their various combinations. The dose of BP aqueous extract was 50 mg kg^{-1} body weight, while the dose of the other materials was 500 mg kg^{-1} body weight. The aqueous extracts were administered orally once daily for eight weeks. Our phytochemical screening detected the presence of flavonoids, tannins, saponins, steroids, and alkaloids in these extracts. The results pertaining to serum levels of glucose, lipids, glycosylated haemoglobin, and antioxidant biomarkers revealed significant improvements after administration of AS, BP + AS, and the mixture of all extracts. Moreover, histological analyses demonstrated the protective effects of these extracts. In conclusion, all tested materials, especially ajwa seeds administered alone and in combination with black pepper extract, exerted significant antihyperlipidemic and weight-stabilising effects in alloxanised diabetic rats. Therefore, these natural substances hold promise as potential herbal medicines for managing diabetes mellitus.

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Introduction

Multiple metabolic diseases affect the health and well-being of people globally. Diabetes mellitus is a chronic metabolic disorder characterised by elevated blood glucose levels resulting either from insufficient insulin production or impaired insulin function. The global prevalence of the disease has reached epidemic proportions, posing significant health and economic burden on societies worldwide (Aziz et al., 2019; WHO, 2021). The current approach to managing diabetes primarily involves synthetic medications aimed at glycaemic control. However, these medications often have adverse effects and limited long-term effectiveness. Consequently, there is a growing interest in exploring natural alternatives, such as medicinal plants and their bioactive compounds, to manage diabetes and its complications (Patergnani et al., 2021).

Black pepper (*Piper nigrum*) and turmeric (*Curcuma longa*) are widely used spices known for their potential therapeutic properties. They possess various bioactive compounds, including alkaloids, flavonoids, and polyphenols, which have been demonstrated to have anti-inflammatory, antioxidant, and anti-diabetic activities (Kalpravidh et al., 2010; Sarfraz et al., 2017). Ajwa dates (*Phoenix dactylifera*) are fruits of a specific cultivar of the date palm known for their nutritional and medicinal values (Robinson et al., 2012). Ajwa date pulp and seeds contain high levels of bioactive compounds, including flavonoids, phenolics, and dietary fibres, which contribute to their antioxidant and anti-diabetic properties (Baliga et al., 2011). Combining these natural ingredients could potentially enhance their individual anti-diabetic effects through synergistic interactions (Sarfraz et al., 2016; Ma et al., 2022).

Oxidative stress occurs as a result of an imbalance between reactive oxygen species (ROS) production and antioxidant defence mechanisms (Shahwar et al., 2012), and it plays an important role in the development and progression of diabetes. Alloxan, a diabetogenic agent, induces oxidative stress by generating ROS and causing damage to pancreatic β-cells. Therefore, targeting oxidative stress through natural antioxidants may be a promising therapeutic approach for diabetes control (Hua et al., 2021; Liu et al., 2022).

Individuals with diabetes must manage their condition to maintain their health, and antidiabetic drugs are commonly used for this purpose. However, there is no universal cure at the moment, and like all medications, the current antidiabetic drugs come with side effects. Hence, the exploration of plant-based alternatives to replace or complement these medications has become important. Recent global studies have focused on natural medicines to reduce the potential side effects associated with conventional antidiabetic drugs (Zein et al., 2020; Mosenzon et al., 2021).

Interestingly, ajwa dates are an economical and nutrition-rich dietary source that has gained global importance (Abdessalem et al., 2019). Similarly, turmeric (*Curcuma longa*) is widely recognised for its medicinal properties. It has been reported to exhibit various biological functions, including the biosynthesis of antioxidants and anti-inflammatory compounds, and demonstrates antimicrobial and anti-atherosclerotic potential (Rivera-Mancía et al., 2018; Pivari et al., 2019). Another noteworthy natural ingredient is black pepper (*Piper nigrum*), which contains an active compound known as piperine. Pharmacological studies have highlighted the diverse biological properties of piperine, attributed to its ability to scavenge free radicals. These activities include cytotoxic, anthelmintic, anti-inflammatory, antidiabetic, hypolipidemic, antioxidant, immunological, hematinic, and hepatoprotective effects (Ahmad et al., 2010).

It was hypothesised in the present study that black pepper, turmeric, ajwa date pulp, seed, and their mixtures would exhibit anti-diabetic effects in alloxan-induced diabetic rats by regulating blood glucose levels, insulin secretion, and lipid profile. Moreover, their antioxidant properties are expected to mitigate oxidative stress. Elucidating the anti-diabetic and antioxidant effects of these natural compounds will provide valuable insights into their potential therapeutic applications in managing diabetes and related complications. Furthermore, this study may contribute to the development of novel phytotherapeutic interventions in diabetes, reducing reliance on synthetic medications and promoting a more holistic approach to diabetes control. The present study was conducted to investigate the anti-diabetic effect of black pepper, turmeric, and ajwa date pulp + seed aqueous extracts and their mixtures as hypolipidemic, antioxidant, and protective agents on the liver and kidney by studying histological changes in alloxanised diabetic rats.

Material and methods

Chemicals and drugs

Glibenclamide tablets (Daonil) were purchased from Sanofi-Aventis PVT. Ltd. Alloxan monohy-

drate was purchased from AppliChem, Ottoweg-4, Germany. All chemicals used in this study were of analytical grade.

Plant material and extract preparation

The experimental materials, including black pepper (BP), turmeric roots (T), and ajwa date pulp (AP) along with the seeds (AS) were purchased from the Faisalabad market, Pakistan. The test materials were thoroughly washed, shade dried, and finely powdered. To prepare aqueous extracts, the plant material (100 g) was soaked in water (1000 ml) for 24 h. The extracts were subsequently filtered and crude active ingredients were obtained.

Research protocol

The experiment was conducted with the prior approval of the Directorate of Research and Advance Studies and with the consent of the Ethics Committee on Animal Experimentation of the University of Agriculture, Faisalabad, Pakistan (Reference No. 5/89/33).

For antidiabetic evaluation, Wistar albino rats were acquired from the Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. The rats were kept in cages of stainless steel and under standard atmospheric conditions (temperature of 24 ± 2 °C and 12 h light/dark cycle). Each cage with a floor area of 1500 cm^2 housed five rats. After one week of acclimatisation, the rats were divided into individual groups. Diabetes was induced by the intraperitoneal injection of alloxan monohydrate (150 mg kg^{-1} body weight). Rats with blood glucose levels above 300 mg dl^{-1} were considered diabetic, as suggested by Yuzefovych et al. (2019). All the rats were provided with water, and a standard diet, and were administered aqueous extracts from the individual test plants separately and in combination with each other, according to the predefined experimental treatments.

Phytochemical screening

Proximate analysis of the test material was carried out using guidelines of the Association of Official Analytical Chemists (AOAC, 1990). For the determination of elements in the samples, an Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Tokyo, Japan) was employed, following the method defined in AOAC. The aqueous extracts of BP, T, AP and AS were subjected to phytochemical analysis using standard methods (Shabi and Kumari, 2014). Quantitative analysis of the plant materials was carried out using high-performance liquid chromatography (HPLC) with a diode array detector (DAD) attached to Discovery C-18 analytical column (Agilent Technologies, Germany). The method was applied with some modifications (Zu et al., 2006). Briefly, chromatographic analysis was carried out using a HIQ SIL C18V reversed-phase column (ø 4.6 mm × 250 mm, KYA TECH Corporation, Japan) packed with 5 µm particles; the mobile phase was methanol-acetonitrile-water (40:15:45, v/v/v) containing 1.0% acetic acid. This mobile phase was filtered through a 0.45 µm membrane filter (Millipore) and then deaerated ultrasonically before use. Piperine, curcumin, rutin, gallic acid, catechin, caffeic acid and quercetin were quantified by DAD following RP-HPLC separation. The flow rate and injection volume were 1.0 ml min^{-1} and 10 µl, respectively. Chromatographic peaks of the analytes were confirmed by comparing their retention time and ultraviolet spectra with the reference standards. Quantification was carried out by integrating the peaks using an external standard method. All chromatographic operations were carried out at ambient temperature.

Oral glucose tolerance test of experimental materials in normal fasted rats

Normal rats were fasted overnight and divided into ten groups of six rats each. Group one served as the control and received distilled water. Group two received 10 mg kg^{-1} Glibenclamide (Sanofi-Aventis PVT. Ltd., Pakistan), while the other groups (3–10) were orally administered an aqueous extract of four natural materials, separately and in combination with each other. The BP aqueous extract was administered at a dose of 50 mg kg^{-1} body weight, while the other materials were administered at a dose of 500 mg kg^{-1} body weight. Thirty minutes after administration of the test substance, all experimental animals were fed orally with glucose (2 g/kg), and tail blood samples were drawn at 0, 30, 60, 90, 120, and 150 min after glucose administration. Blood glucose levels were determined using glucose-peroxide reagent strips and the data recorded were statistically analysed to evaluate significant differences between the different groups.

Hypoglycaemic effect of aqueous extract of test materials in alloxan diabetic rats

The antihyperglycaemic effects of the aqueous extracts in hyperglycaemic rats fasted overnight was determined in the previously formed groups administered with the experimental materials. Blood glucose levels were measured after 0, 2, 4, 6, 8, and 24 h using glucose-peroxidase reagent strips by collecting blood samples from the tail vein.

Experimental design and sample collection

Wistar albino rats (396 male and female adults) weighing 180–200 g at two weeks of age were divided into eleven groups, with each group composed of eighteen males and eighteen females. The experiment consisted of the following groups: a normal control (non-diabetic), a diabetic control (positive control), a group receiving Glibenclamide (10 mg kg^{-1}), and groups receiving aqueous extracts of BP, T, AP, and AS, applied either alone or in combination with each other, as well as a group receiving all four materials combined. The dose of BP aqueous extract was 50 mg kg^{-1} body weight, while the dose of the other materials was 500 mg kg^{-1} body weight. The aqueous extracts were administered orally by gastric tube once daily for eight weeks. Blood samples were collected in week 0, 4, and 8 of the experiment and serum was separated for the determination of biochemical parameters. The animals were euthanized by carbon dioxide inhalation and dissected to collect organ samples. The liver and kidney were evaluated histopathologically to examine the appearance of structures of interest.

Physical parameters

Body weight of each animal was recorded weekly throughout the experimental period using a digital weighing balance to check for any significant differences between the start and end of the experiment.

Feed intake of the rats was recorded daily. Before offering feed to the animals, they were weighed, as was weighed the leftover feed from the previous day to determine the daily food intake of different groups to observe any changes in feed consumption.

Water intake was recorded daily in each group to analyse any significant differences between individual experimental groups.

Biochemical and histopathological analyses

Blood glucose levels of the rats were determined using a commercially available On-Call® Ez II (SN 303S0014E09) glucometer (Acon Diabetes Care International, California, USA). Blood glucose was obtained from the tail snip and the data were recorded for further evaluation. Serum aryl esterase activity was determined according to the method of Juretic et al. (2006). Total protein was determined by the biuret method using the 83LS100-60 total protein test kit (Span Diagnostics Ltd., India) (Gornall et al., 1949). Albumin levels were assessed by the method described by Corcoran and Durnan (1977) using the 84LS100-60 test kit (Span Diagnostics Ltd., India). Globulin levels were determined by subtracting the albumin protein level from the total protein level. For the measurements of the activity of liver enzymes, namely, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), a commercially available BT294Q kit (Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom) was used. The total bilirubin in the sample was determined by the Jendrassik-Grof method. Serum creatinine levels were measured spectrophotometrically using a commercially available Creatinine Assay MAK080 Kit (Creatinine Jaffe Ecoline® diagnostic kit Merck, Holzheim, Germany). Urea levels were determined by the urease-GLDH (glutathione dehydrogenase) enzymatic UV method using a commercially available kit (Breuer and Breuer Diagnostics, Ref# 04460715 190, Italy). Serum uric acid was determined by using a commercially available kit (Innoline Merck, Ref#1692, USA). The data recorded for each parameter was statistically analysed to check for differences between treatment means at a significance level of $\alpha = 0.05$. Histopathology of the liver and kidney was performed following the procedure of Bancroft and Gamble (2007).

Statistical analysis

The data collected were statistically analysed using the analysis of variance (ANOVA) and the Statistix software package (version 8.1). Subsequently, treatment means were compared using Duncan's multiple range test (DMRT) at the 5% probability level, as suggested by Snedecor and Cochron (1989).

Results

The proximate composition of all the natural materials tested is shown in Table 1. The results showed that all aqueous extracts were a good source of fibre and protein. The concentration of minerals in the tested materials is shown in Table 2.

Calcium concentration was the highest in AS at 191.8 ± 0.69 mg 100 g^{-1} dry matter (DM), while the lowest in T at 180.0 ± 1.92 mg 100 g^{-1} DM. Iron concentration was high in AP 3.60 ± 1.22 mg 100 g^{-1} DM, followed in descending order by BP, AS and T (2.80 ± 1.12, 1.60 ± 1.10 and 1.50 ± 1.87 mg 100 g^{-1} dry weight, respectively). The results of the qualitative phytochemical analysis of the aqueous extracts of BP, T, AP and AS are shown in Table 3.

All sample extracts showed the presence of phenols, carbohydrates, and proteins. Additionally, the HPLC profile revealed specific compounds in each extract. BP contained piperine (40 mg/g),

Table 1. Proximate composition of natural materials used in the study, %

Parameter	Black pepper	Turmeric	Ajwa pulp	Ajwa seeds
Moisture	11.55 ± 0.75^{cd}	10.85 ± 0.02^{d}	18.80 ± 0.46^{b}	05.45 ± 0.38^{h}
Crude protein	08.65 ± 0.21^{ef}	09.12 ± 0.34^{e}	02.60 ± 0.30^{j}	05.80 ± 0.30^{h}
Crude fat content	12.78 ± 0.02^{c}	03.76 ± 0.01^{ij}	03.20 ± 0.10^{j}	11.70 ± 0.20^{cd}
Crude fibre content	05.20 ± 0.40^{h}	05.62 ± 0.25^{h}	07.60 ± 0.20^{fg}	48.90 ± 0.20^{a}
Ash content	07.44 ± 0.03^{fg}	07.21 ± 0.30^{g}	04.60 ± 0.04^{hi}	03.40 ± 0.06^{ij}

data are presented as mean values ± SE; SE – standard error, $^{a–j}$ – mean values in columns and rows with a different superscript are significantly different at $P \leq 0.05$

Table 2. Mineral composition of natural materials used in the study, mg 100 g^{-1}

Mineral	Black pepper	Turmeric	Ajwa pulp	Ajwa seeds
Calcium	181.0 ± 2.31^{c}	180.0 ± 1.92^{c}	189.1 ± 1.65^{b}	191.8 ± 0.69^{b}
Copper	00.24 ± 0.81^{g}	00.57 ± 0.12^{fg}	00.21 ± 0.10^{g}	00.60 ± 0.21^{fg}
Iron	02.80 ± 1.12^{fg}	01.50 ± 1.87^{fg}	03.60 ± 1.22^{f}	01.60 ± 1.10^{fg}
Magnesium	230.0 ± 1.42^{a}	190.0 ± 1.53^{b}	150.0 ± 0.71^{d}	180.0 ± 1.22^{c}
Manganese	01.80 ± 0.62^{fg}	07.83 ± 0.43^{e}	00.29 ± 0.11^{g}	01.70 ± 0.15^{fg}
Zinc	01.45 ± 0.54^{fg}	03.41 ± 0.42^{fg}	01.42 ± 0.19^{fg}	01.40 ± 0.49^{fg}

data are presented as mean values ± SE; SE – standard error, $^{a–g}$ – mean values in columns and rows with a different superscript are significantly different at $P \leq 0.05$

Table 3. Qualitative analysis of aqueous extracts of natural materials used in the study

Phytochemical constituents	Observation, present/absent			
	black pepper	turmeric	ajwa pulp	ajwa seed
Flavonoids	-	+	+	+
Tannins	+	-	+	-
Alkaloids	+	+	-	-
Phenols	+	+	+	+
Saponins	-	-	+	-
Proteins	+	+	+	+
Fixed oils	-	+	+	+
Carbohydrate	+	+	+	+
Glycosides	-	+	+	-
Steroids	-	+	+	+

(+) present, (-) absent

T contained curcumin (0.882 mg/g), AP contained rutin (0.007 mg/g) and AS contained gallic acid and quercetin (0.011 and 0.034 mg/g, respectively). Furthermore, catechin (0.0078 and 0.077 mg/g) andcaffeic acid (0.006 and 0.048 mg/g) were detected in both AP and AS, respectively (Table 4).

The mean body weight, feed, and water intake in individual treatment groups from week 1 to week 8 are presented in Figure 1A–C and Tables 5–7. The body weight of diabetic rats treated with different aqueous extracts was stable but significantly ($P \leq 0.05$) increased in comparison to untreated diabetic rats. However, when compared to the normal control group, the difference was not significant. The feed intake of the normal and untreated diabetic control group showed non-significant changes until week 7. However, in the groups treated with individual extracts (T, AP, and AS) or a combination of BP + AP, there was a significant ($P \leq 0.05$) decline in feed intake from week 6 to week 8. Similarly, the groups treated with BP + AS and the combination of all four natural materials also showed a decrease in feed intake from week 3 to week 8. Regarding water intake, all the different aqueous extract-treated groups showed significant ($P \leq 0.05$) differences from week 1 to week 8.

The oral glucose tolerance test was performed to evaluate the hypoglycaemic effect of the aqueous extracts in glucose-loaded rats. The mean blood glucose levels in individual treatments at different time intervals are given in Figure 2A. Figure 2B clearly indicates that the treatments with all four natural materials produced a non-significant ($P > 0.05$) decline in glucose levels after 2 h, and a significant decrease was observed after 8 h.

Table 4. High-performance liquid chromatography (HPLC) analysis of compounds in black pepper, turmeric, ajwa pulp, and ajwa seeds, mg g^{-1} dry weight

Sample	Piperine	Curcumin	Rutin	Gallic acid	Catechin	Caffeic acid	Quercetin
Black pepper	40	-	-	-	-	-	-
Turmeric	-	0.882	-	-	-		-
Ajwa pulp	-	-	0.007	-	0.0078	0.006	-
Ajwa seeds	-	-	-	0.011	0.077	0.048	0.034

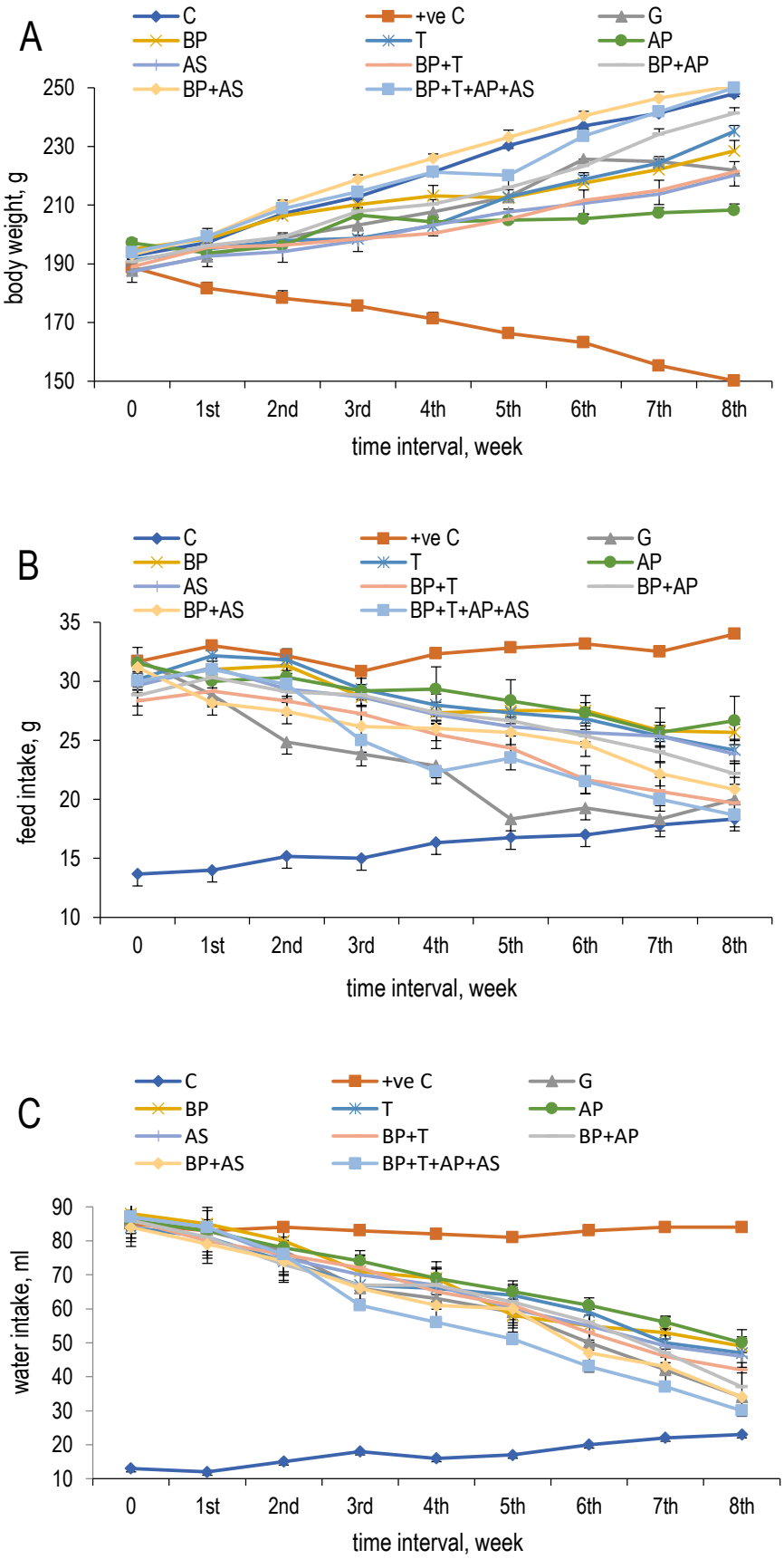

Figure 1. Effects of aqueous extracts of the test materials on body weight (A), feed intake (B), and water intake (C) (ml ± SE) of diabetic rats in consecutive weeks

SE – standard error, C – control, +ve C – positive diabetic control, G – glibenclamide, BP – black pepper, T – turmeric, AP – ajwa pulp, AS – ajwa seed

Table 5. Body weight of diabetic rats treated with aqueous extracts of black pepper, turmeric, ajwa pulp, and ajwa seeds in consecutive weeks, g

Treatment groups	Weeks								
	0	1	2	3	4	5	6	7	8
Control	192.67 ± 2.35	197.33 ± 1.83^{NS}	207.11 ± 2.05*	212.83 ± 2.30*	221.33 ± 1.97**	230.33 ± 1.45**	237.01 ± 1.67**	241.33 ± 1.87**	248.00 ± 2.94**
Positive control	188.83 ± 2.14	181.66 ± 2.07^{NS}	178.33 ± 2.51*	175.67 ± 1.72**	171.33 ± 2.17**	166.33 ± 1.90**	163.18 ± 2.05**	155.33 ± 1.56**	150.17 ± 1.51**
10 mg kg^{-1} G	187.67 ± 2.44	192.50 ± 2.32^{NS}	198.84 ± 1.98*	203.16 ± 1.27*	207.66 ± 1.30*	212.83 ± 2.41**	225.66 ± 1.60**	224.83 ± 1.51**	222.00 ± 2.71**
50 mg kg^{-1} BP	195.00 ± 1.59	198.50 ± 2.23^{NS}	206.34 ± 2.03*	210.16 ± 1.68**	213.17 ± 2.01**	212.50 ± 2.44**	217.50 ± 1.33**	222.17 ± 1.98**	228.50 ± 1.43**
500 mg kg^{-1} T	191.17 ± 1.47	195.17 ± 2.37^{NS}	197.83 ± 2.01*	198.66 ± 1.05*	203.00 ± 0.93*	212.83 ± 2.41**	218.87 ± 1.35**	224.33 ± 2.23**	235.17 ± 1.99**
500 mg kg^{-1} AP	197.17 ± 0.94	193.67 ± 2.12^{NS}	196.16 ± 1.98^{NS}	206.67 ± 1.54*	204.33 ± 1.76*	204.83 ± 2.74*	205.33 ± 1.54*	207.33 ± 1.83*	208.33 ± 2.07**
500 mg kg^{-1} AS	187.33 ± 1.05	192.67 ± 2.28^{NS}	194.17 ± 1.62*	197.83 ± 0.83*	203.17 ± 0.83*	207.67 ± 2.55**	210.67 ± 1.80**	213.83 ± 1.42**	220.17 ± 2.64**
500 mg kg^{-1} BP + T	189.00 ± 0.93	195.50 ± 2.43^{NS}	196.33 ± 1.89*	198.50 ± 0.76*	200.33 ± 2.83*	205.16 ± 2.46**	211.66 ± 2.17**	215.00 ± 2.86**	221.33 ± 1.98**
500 mg kg^{-1} BP + AP	190.67 ± 0.88	196.17 ± 2.46^{NS}	199.16 ± 1.40*	207.83 ± 1.37*	210.33 ± 1.54**	216.00 ± 2.93**	223.33 ± 2.34**	234.17 ± 1.85**	241.33 ± 1.87**
500 mg kg^{-1} BP + AS	193.17 ± 2.59	199.33 ± 1.64^{NS}	210.33 ± 1.35*	218.83 ± 1.49**	226.00 ± 1.46**	233.17 ± 2.42**	240.50 ± 1.47**	246.50 ± 2.14**	250.67 ± 2.82**
500 mg kg^{-1} BP + T + AP + AS	194.00 ± 1.98	199.33 ± 2.18^{NS}	208.83 ± 1.53*	214.50 ± 1.89**	221.33 ± 1.97**	220.17 ± 2.67**	233.50 ± 1.97**	241.83 ± 1.13**	250.00 ± 2.69**

G – glibenclamide, BP – black pepper, T – turmeric, AP – ajwa pulp, AS – ajwa seed; data are presented as mean values ± standard error; * indicates significant difference compared to week zero ($P \leq 0.05$); ** indicates significant difference compared to week zero ($P \leq 0.001$); ^{NS} indicates non-significant difference compared to week zero ($P \leq 0.05$)

Table 6. Feed intake of diabetic rats treated with aqueous extracts of black pepper, turmeric, ajwa pulp, and ajwa seeds in consecutive weeks, g day^{-1}

Treatment groups	Weeks								
	0	1	2	3	4	5	6	7	8
Control	13.66 ± 0.88	14.00 ± 1.46^{NS}	15.11 ± 1.45^{NS}	15.00 ± 1.78^{NS}	16.31 ± 1.28^{NS}	16.76 ± 1.11^{NS}	17.01 ± 1.46^{NS}	17.83 ± 1.18^{NS}	18.33 ± 0.88*
Positive control	31.83 ± 2.05	33.00 ± 2.05^{NS}	32.13 ± 1.01^{NS}	30.83 ± 1.07^{NS}	32.33 ± 1.17^{NS}	32.83 ± 2.41^{NS}	33.18 ± 1.98^{NS}	32.50 ± 1.63^{NS}	34.00 ± 1.17^{NS}
10 mg kg^{-1} G	31.96 ± 1.25	28.99 ± 1.63^{NS}	24.84 ± 1.10*	23.83 ± 1.42**	22.83 ± 1.72**	18.33 ± 1.68**	19.26 ± 1.03**	18.33 ± 1.22**	20.00 ± 1.20**
50 mg kg^{-1} BP	30.00 ± 1.56	31.00 ± 1.57^{NS}	31.44 ± 1.33^{NS}	28.66 ± 1.23^{NS}	27.33 ± 1.18^{NS}	27.50 ± 1.47^{NS}	27.50 ± 1.21^{NS}	25.83 ± 1.04*	25.67 ± 0.89**
500 mg kg^{-1} T	30.10 ± 1.46	32.16 ± 1.42^{NS}	31.98 ± 2.21^{NS}	29.33 ± 1.36^{NS}	28.00 ± 0.58^{NS}	27.33 ± 1.26^{NS}	26.83 ± 2.11*	25.33 ± 1.22*	24.16 ± 1.91**
500 mg kg^{-1} AP	31.46 ± 1.51	30.00 ± 1.46^{NS}	30.19 ± 1.27^{NS}	29.16 ± 1.25^{NS}	29.33 ± 1.76^{NS}	28.33 ± 1.12^{NS}	27.33 ± 1.76*	25.66 ± 2.06*	26.66 ± 2.09**
500 mg kg^{-1} AS	29.16 ± 1.70	31.16 ± 1.14^{NS}	29.21 ± 1.48^{NS}	28.66 ± 1.62^{NS}	27.17 ± 1.58^{NS}	26.16 ± 1.27^{NS}	25.65 ± 2.07*	25.33 ± 1.25*	23.83 ± 1.30**
500 mg kg^{-1} BP + T	28.36 ± 1.66	29.16 ± 1.42^{NS}	28.38 ± 1.09^{NS}	27.26 ± 1.12^{NS}	25.50 ± 1.17^{NS}	24.33 ± 1.36*	21.67 ± 2.01**	20.66 ± 2.06**	19.66 ± 1.42**
500 mg kg^{-1} BP + AP	28.86 ± 1.58	30.33 ± 1.47^{NS}	29.08 ± 1.18^{NS}	28.83 ± 1.40^{NS}	27.33 ± 1.84^{NS}	26.66 ± 1.20^{NS}	25.33 ± 1.34*	24.00 ± 1.76*	22.16 ± 1.91**
500 mg kg^{-1} BP + AS	31.16 ± 1.25	28.16 ± 1.51^{NS}	27.73 ± 1.28^{NS}	26.16 ± 1.17*	26.00 ± 1.00*	25.66 ± 1.63*	24.66 ± 2.04*	22.16 ± 1.72**	20.83 ± 1.92**
500 mg kg^{-1} BP + T + AP + AS	30.00 ± 1.46	31.00 ± 1.46^{NS}	29.89 ± 1.29^{NS}	25.00 ± 1.46*	22.33 ± 1.60**	23.50 ± 1.11**	21.50 ± 1.29**	20.00 ± 1.55**	18.67 ± 1.45**

G – glibenclamide, BP – black pepper, T – turmeric, AP – ajwa pulp, AS – ajwa seed; data are presented as mean values ± standard error; * indicates significant difference compared to week zero ($P \leq 0.05$); ** indicates significant difference compared to week zero ($P \leq 0.001$); ^{NS} indicates non-significant difference compared to week zero ($P \leq 0.05$)

Table 7. Water intake of diabetic rats treated with aqueous extracts of black pepper, turmeric, ajwa pulp, and ajwa seeds in consecutive weeks, ml day^{-1}

Treatment groups	Weeks							
	0	1	2	3	4	5	6	7	8
Control	13.00 ± 1.71	12.00 ± 0.86^{NS}	15.00 ± 1.45^{NS}	18.00 ± 1.55^{NS}	16.00 ± 1.18^{NS}	17.00 ± 1.21^{NS}	20.00 ± 1.26*	22.00 ± 1.76*	23.00 ± 1.76*
Positive control	85.00 ± 3.70	83.00 ± 3.40^{NS}	84.00 ± 1.41^{NS}	83.00 ± 2.56^{NS}	82.00 ± 1.55^{NS}	81.00 ± 1.95^{NS}	83.00 ± 1.58^{NS}	84.00 ± 0.58^{NS}	84.00 ± 1.55^{NS}
10 mg kg^{-1} G	86.00 ± 3.55	83.00 ± 3.40*	77.00 ± 1.30**	66.00 ± 1.76**	63.00 ± 1.17**	59.00 ± 2.16**	50.00 ± 1.03**	42.00 ± 1.17**	34.00 ± 1.76**
50 mg kg^{-1} BP	88.00 ± 2.08	85.00 ± 3.70*	80.00 ± 1.33*	71.00 ± 1.01**	69.00 ± 2.69**	58.00 ± 1.29**	55.00 ± 1.21**	53.00 ± 2.12**	49.00 ± 1.55**
500 mg kg^{-1} T	84.00 ± 2.08	81.00 ± 1.95*	74.00 ± 2.21**	67.00 ± 1.77**	66.00 ± 2.35**	64.00 ± 1.73**	59.00 ± 1.11**	50.00 ± 1.17**	47.00 ± 2.12**
500 mg kg^{-1} AP	86.00 ± 3.55	83.00 ± 1.57*	78.00 ± 1.27*	74.00 ± 1.55**	69.00 ± 2.69**	65.00 ± 2.05**	61.00 ± 1.76**	56.00 ± 1.55**	50.00 ± 1.76**
500 mg kg^{-1} AS	87.00 ± 3.55	84.00 ± 2.08*	75.00 ± 1.48**	70.00 ± 1.17**	67.00 ± 2.35**	60.00 ± 2.16**	55.00 ± 1.67**	49.00 ± 1.55**	46.00 ± 1.54**
500 mg kg^{-1} BP + T	86.00 ± 3.55	80.00 ± 3.40*	76.00 ± 1.29**	72.00 ± 1.93**	65.00 ± 1.17**	61.00 ± 1.73**	53.00 ± 1.41**	46.00 ± 2.12**	42.00 ± 2.10**
500 mg kg^{-1} BP + AP	85.00 ± 3.61	81.00 ± 1.65*	73.00 ± 1.18**	67.00 ± 1.76**	67.00 ± 2.35**	62.00 ± 2.35**	56.00 ± 1.34**	47.00 ± 2.11**	37.00 ± 1.56**
500 mg kg^{-1} BP + AS	84.00 ± 3.44	79.00 ± 1.95*	74.00 ± 1.28**	66.00 ± 1.76**	61.00 ± 1.76**	59.00 ± 1.29**	47.00 ± 1.45**	43.00 ± 1.18**	34.00 ± 1.74**
500 mg kg^{-1} BP + T + AP + AS	87.00 ± 2.95	84.00 ± 2.08*	76.00 ± 1.29**	61.00 ± 1.55**	56.00 ± 2.69**	51.00 ± 2.05**	43.00 ± 1.29**	37.00 ± 2.56**	30.00 ± 1.57**

G – glibenclamide, BP – black pepper, T – turmeric, AP – ajwa pulp, AS – ajwa seed; data are presented as mean values ± standard error; * indicates significant difference compared to week zero ($P \leq 0.05$); ** indicates significant difference compared to week zero ($P \leq 0.001$); ^{NS} indicates non-significant difference compared to week zero ($P \leq 0.05$)

Similarly, different combinations of the extracts showed highly significant glucose-lowering capabilities up to 24 h, surpassing the glucose-lowering effect of the standard drug, glibenclamide. The mean blood glucose levels in different treatments over consecutive weeks are illustrated in Figure 2C. The hypoglycaemic effects were highly significant ($P \leq 0.05$) at weeks 4 and 8 in the group treated with a mixture of BP + T + AP + AS. In addition, it was observed that aqueous extracts of all tested materials, whether administered separately or in different combinations, triggered a significant decrease in serum glucose in alloxanised hyperglycaemic rats compared to normal control rats (Figure 2D).

A significant ($P \leq 0.05$) increase in aryl esterase levels was observed in diabetic untreated rats compared to healthy animals. Treatments resulted in a significant reduction in aryl esterase levels, as shown in Table 8. In this study, the overall mean serum aryl esterase level was highest in the ajwa seeds-treated group at the end of the experiment. Serum levels of total protein, albumin and globulin in the group of diabetic rats decreased significantly compared to the normal control group. Overall, total protein, albumin, and globulin levels were highest in the control group and lowest in the positive control group, while a non-significant change was recorded between the different treatment groups (Table 8).

Table 8. Serum arylesterase, total protein, albumin, and globulin levels in diabetic rats administered aqueous extracts of natural materials after eight weeks

Treatment groups	Arylesterase, U ml^{-1} min^{-1}	Total protein, g dl^{-1}	Albumin, g dl^{-1}	Globulin, g dl^{-1}
Control	156.38 ± 0.28^{a}	7.27 ± 0.05^{a}	4.73 ± 0.02^{a}	2.81 ± 0.09^{a}
Positive control	41.14 ± 0.42^{g}	3.96 ± 0.08^{c}	2.92 ± 0.04^{c}	1.26 ± 0.04^{c}
10 mg kg^{-1} G	73.22 ± 5.46^{f}	5.61 ± 0.14^{b}	4.12 ± 0.09^{b}	1.94 ± 0.10^{b}
50 mg kg^{-1} BP	76.91 ± 5.19^{e}	5.15 ± 0.12^{b}	3.75 ± 0.07^{b}	1.75 ± 0.07^{b}
500 mg kg^{-1} T	82.94 ± 5.38^{d}	5.40 ± 0.13^{b}	3.94 ± 0.06^{b}	1.76 ± 0.07^{b}
500 mg kg^{-1} AP	80.47 ± 5.16^{de}	5.34 ± 0.11^{b}	3.91 ± 0.05^{b}	1.77 ± 0.08^{b}
500 mg kg^{-1} AS	92.02 ± 4.81^{b}	5.13 ± 0.12^{b}	3.83 ± 0.06^{b}	1.64 ± 0.08^{bc}
500 mg kg^{-1} BP + T	76.94 ± 5.80^{e}	5.48 ± 0.14^{b}	3.80 ± 0.08^{b}	1.67 ± 0.07^{b}
500 mg kg^{-1} BP + AP	75.47 ± 5.98^{ef}	5.36 ± 0.16^{b}	3.68 ± 0.11^{b}	1.68 ± 0.08^{b}
500 mg kg^{-1} BP + AS	88.69 ± 6.30^{bc}	5.54 ± 0.15^{b}	4.08 ± 0.06^{b}	1.62 ± 0.09^{bc}
500 mg kg^{-1} BP + T + AP+ AS	87.27 ± 6.60^{c}	5.61 ± 0.014^{b}	4.12 ± 0.08^{b}	1.66 ± 0.08^{b}

G – glibenclamide, BP – black pepper, T – turmeric, AP – ajwa pulp, AS – ajwa seed; data are presented as mean values ± standard error, $^{a\text{-}f}$ – mean values within a row or column with a different superscript are significantly different at $P \leq 0.05$

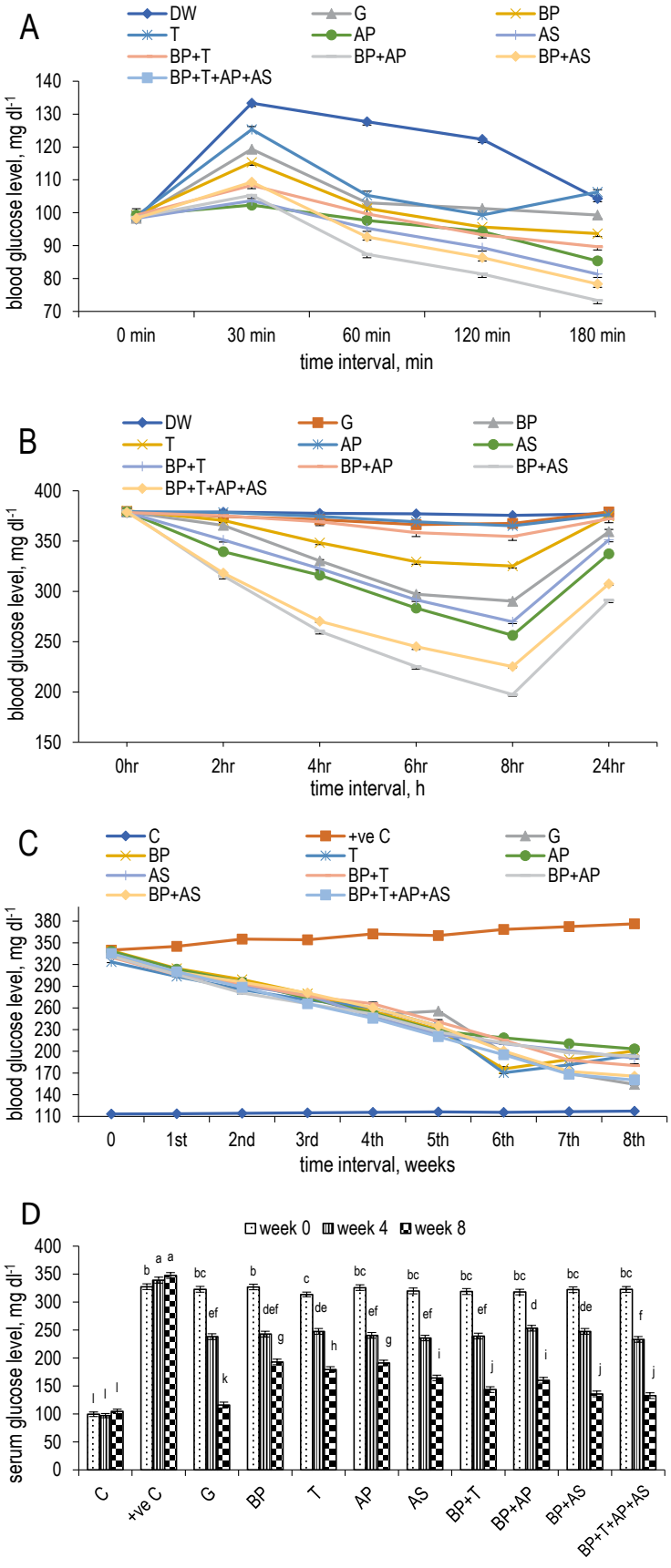

Figure 2. Effects of black pepper, ajwa pulp, turmeric, and ajwa seed aqueous extracts on (A) oral glucose tolerance test, (B) blood glucose level, (C) fasting blood glucose level, (D) serum glucose level (mg dl-1) in diabetic rats after 0, 4 and 8 weeks

C – control, +ve C – positive diabetic control, G – glibenclamide, BP – black pepper, T – turmeric, AP – ajwa pulp, AS – ajwa seed

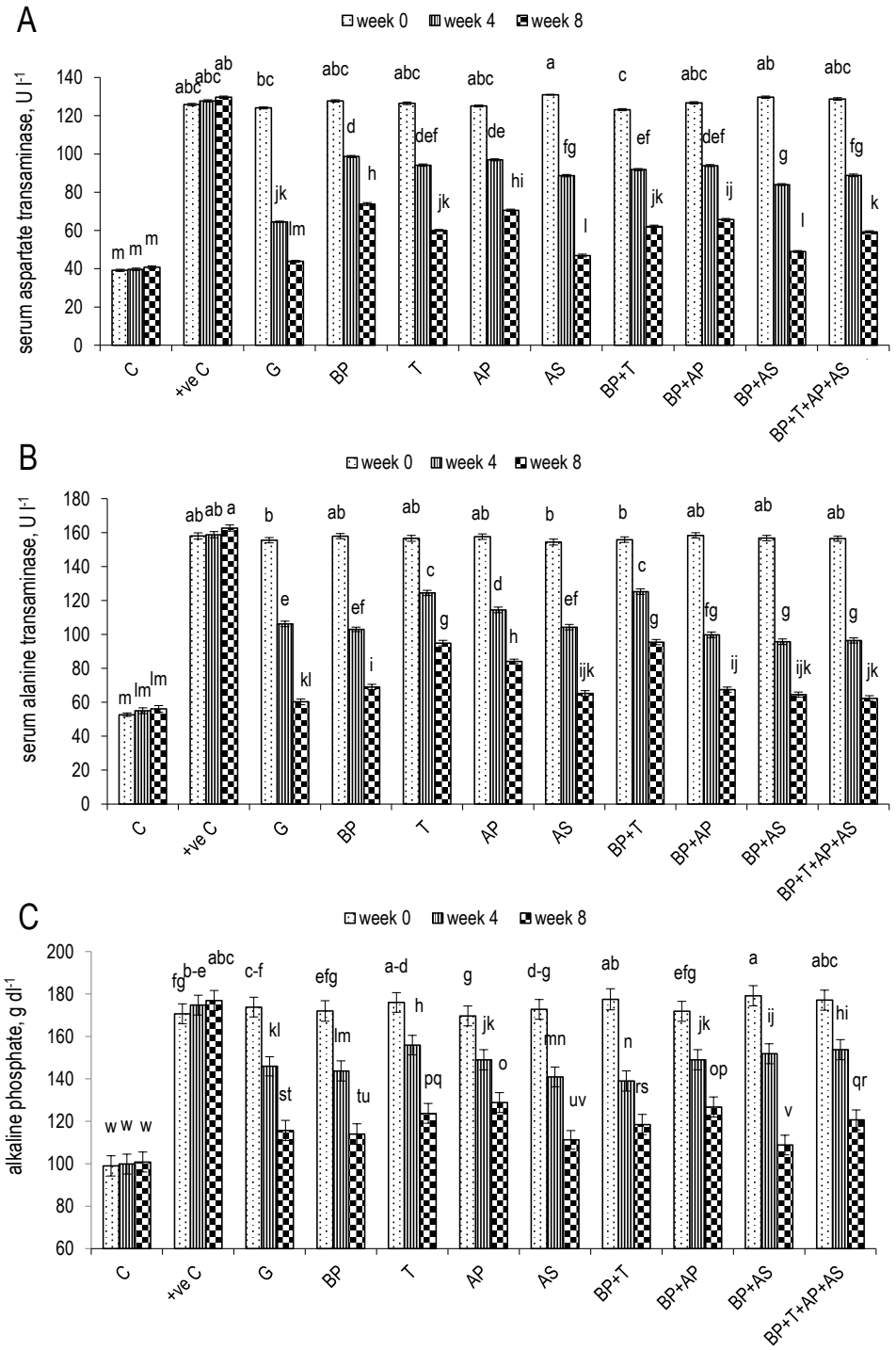

Figure 3. Effects of aqueous extracts of black pepper, turmeric, ajwa pulp, and ajwa seeds on serum AST(A), ALT (B), and ALP (C) activity levels in diabetic rats after 0, 4, and 8 weeks

AST – aspartate aminotransferase, ALT – alanine transaminase, ALP – alkaline phosphatase, C – control, +ve C – positive control-diabetic, G – glibenclamide, BP – black pepper, T – turmeric, AP – ajwa pulp, AS – ajwa seeds; bars sharing the same letter(s) do not differ significantly at $P \leq 0.05$

Mean values of serum AST, ALT, and ALP activities in individual treatment groups at successive weeks are plotted in Figure 3A–C. The diabetic groups treated with natural materials and their different combinations showed an improvement in ALT, AST, and ALP activity levels, but their values changed significantly ($P \leq 0.05$) in comparison to the normal rat group.

A significant rise in bilirubin and creatinine concentrations were detected in the diabetic untreated group, with non-significant differences observed at different weeks. Overall mean serum

Table 9. Serum bilirubin, creatinine, urea, and uric acid levels in diabetic rats administered aqueous extracts of natural materials after eight weeks, mg dl^{-1}

Treatment groups	Bilirubin	Creatinine	Urea	Uric acid
Control	0.89 ± 0.0^{f}	0.22 ± 0.01^{h}	28.11 ± 0.23^{d}	3.18 ± 0.76^{c}
Positive control	1.82 ± 0.01^{a}	1.94 ± 0.06^{a}	65.92 ± 0.88^{a}	4.18 ± 1.06^{a}
10 mg kg^{-1} G	1.33 ± 0.06^{cd}	0.95 ± 0.09^{g}	45.58 ± 2.23^{b}	3.53 ± 0.85^{b}
50 mg kg^{-1} BP	1.37 ± 0.05^{b}	1.23 ± 0.06^{b}	49.89 ± 1.77^{bc}	3.74 ± 0.88^{ab}
500 mg kg^{-1} T	1.36 ± 0.05^{bc}	0.96 ± 0.08^{fg}	48.86 ± 1.76^{bc}	3.79 ± 0.89^{ab}
500 mg kg^{-1} AP	1.38 ± 0.05^{b}	1.03 ± 0.08^{d}	47.86 ± 1.53^{bc}	3.77 ± 0.88^{ab}
500 mg kg^{-1} AS	1.36 ± 0.05^{b}	1.01 ± 0.08^{de}	47.97 ± 1.69^{bc}	3.72 ± 0.85^{ab}
500 mg kg^{-1} BP + T	1.36 ± 0.05^{b}	1.16 ± 0.06^{c}	46.61 ± 1.61^{bc}	3.74 ± 0.88^{ab}
500 mg kg^{-1} BP + AP	1.35 ± 0.06^{bc}	1.02 ± 0.07^{de}	46.02 ± 1.90^{bc}	3.73 ± 0.88^{ab}
500 mg kg^{-1} BP + AS	1.32 ± 0.05^{d}	1.01 ± 0.08^{de}	44.83 ± 2.00^{bc}	3.79 ± 0.86^{ab}
500 mg kg^{-1} BP + T + AP+ AS	1.29 ± 0.06^{e}	0.99 ± 0.08^{ef}	43.20 ± 2.19^{c}	3.84 ± 0.86^{ab}

G – glibenclamide, BP – black pepper, T – turmeric, AP – ajwa pulp, AS – ajwa seed; data are presented as mean values ± standard error, $^{a–g}$ – mean values within a row or column with a different superscript are significantly different at $P \leq 0.05$

bilirubin and creatinine levels in the group administered all four natural materials combined showed similar effects to glibenclamide, while still demonstrating a significant change ($P \leq 0.05$) compared to the diabetic untreated group (Table 9).

Table 9 clearly demonstrates that untreated diabetic rats had significantly ($P \leq 0.05$) elevated serum urea levels accompanied by slightly increased uric acid levels in comparison to the normal control group. The diabetic groups administered natural materials either separately or their combinations showed significantly reduced urea levels ($P \leq 0.05$) after 4 and 8 weeks. However, the latter treatment did not have a significant impact on uric acid levels compared to the other treatment groups, the standard drug glibenclamide, and the positive control group. The overall mean serum urea level was maximal ($P \leq 0.05$) in the positive control group and minimal ($P \leq 0.05$) in the group that received all four plant materials combined.

Histopathological analysis

The histological appearance of the liver is demonstrated in Figure 4. Microscopically, the liver from the normal control group had normal histological structure of the hepatic lobule and portal vein without any alterations (A); the micrograph showed that hepatocytes were arranged in branching cords, separated by blood sinusoids and radiated from the central vein. Liver tissues of diabetic rats showed marked activation of Kupffer cells, sinusoidal leucocytosis and apoptosis of hepatocytes, marked dilatation and congestion of the central vein with necrosis of sporadic hepatocytes (B). Histopathological examination of diabetic rats treated with glibenclamide revealed homogeneous normal hepatic lobules and hepatocytes (C). Liver tissues from diabetic rats in response to all aqueous extracts showed an apparently normal histological structure, except for a slight activation of Kupffer cells (D–G). Liver tissues from diabetic rats treated with BP + T and BP + AP showed Kupffer cell activation and slight congestion of hepatic sinusoids with binucleation of hepatocytes (H–I). Liver sections of diabetic rats treated with BP + AS, BP + T + AP + AS showed Kupffer cell activation, but no significant histopathological alterations were observed (J, K).

Figure 5 illustrates the histological appearance of the kidney of diabetic rats after treatments. The histological appearance of the normal control group reveals normal appearance of glomeruli and tubules (A). Alterations in renal tissues (vessel congestion and interstitial haemorrhages) together with damaged glomeruli and infiltration of red blood cells were observed after induction of diabetes (B). The glibenclamide-treated group showed less pathological lesions and nearly normal architecture of cells with normal glomeruli (C). Minor, cloudy swelling in tubules was observed in groups treated with AS, T, BP, and AP (D–G). Groups that received different combinations of BP with the other materials demonstrated a reversal of this pathological damage, as evidenced by cell regeneration and restoration of normal glomerular structure (H–K).

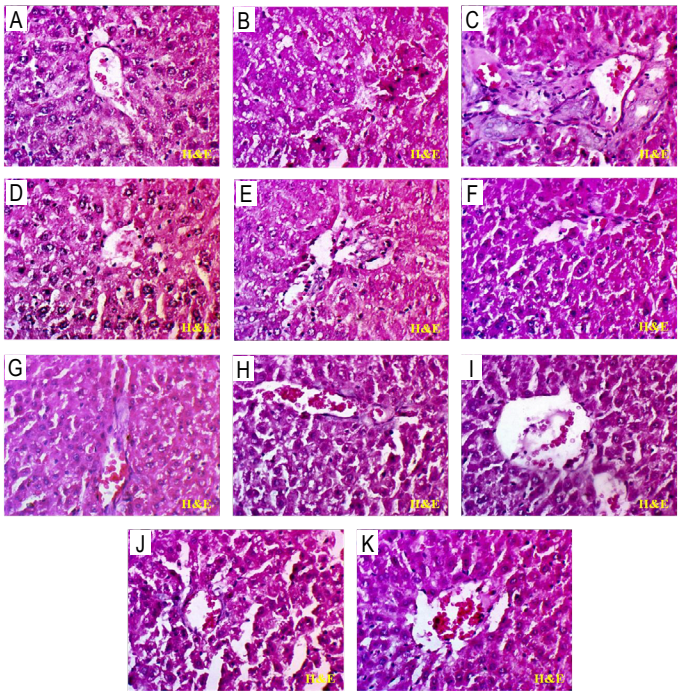

Figure 4. Haematoxylin and eosin (H&E) stained liver sections

(A) control – liver with central vein and radiating cords of hepatocytes; (B) positive control – liver with degenerated hepatocytes and areas of cell necrosis and bile duct; (C) diabetic rats treated with glibenclamide – normal portal tract with no visible lesions; (D–G) diabetic rats treated with black pepper (BP), turmeric (T), ajwa pulp (AP), and ajwa seeds (AS) extracts – oedematous and congested portal vein with minor haemorrhages; H–K) diabetic rats treated with different combinations of extracts (BP + T, BP + AP, BP + AS, BP + T + AP + AS) – bile duct, hepatocytes, and blood vessels with normal architecture; magnification 400x

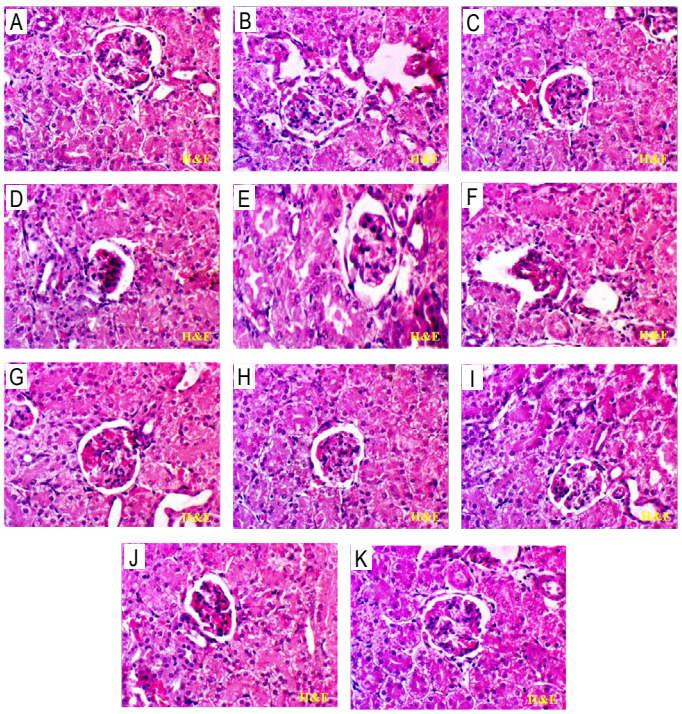

Figure 5. Kidney sections stained with haematoxylin and eosin (H&E)

(A) control – kidney showing normal glomerular architecture and compact tissue; (B) positive control – kidney with damaged glomerulus and profuse infiltration by inflammatory cells; (C) glibenclamide group – kidney with compact cell architecture and glomeruli; (D–G) diabetic rats administered black pepper (BP), turmeric (T), ajwa pulp (AP), and ajwa seeds (AS) extracts – kidney with Bowman's space, glomerulus with dilated loops, suffused red blood cells and few inflammatory cells; (H–K) diabetic rats treated with different combinations of test material extracts (BP + T, BP + AP, BP + AS, BP + T + AP + AS) – normal glomerular structure and compact kidney architecture; magnification 400x

Discussion

The increasing prevalence of diabetes has led to the search for novel and bioactive compounds present in plant extracts, which have been utilised in traditional medicine since ancient times. While screening major phytochemicals can be useful, it may not always account for the valuable effects of the extract. In the present study, proximate analysis of the aqueous extracts of BP, T, AP, and AS indicated that the fat content in BP and AS was high. Moreover, the dietary fibre content was also high in AS in contrast to AP, T, and BP. This was consistent with a report by Al-Farsi and Lee (2008), which stated that water extracts of date pits were rich in dietary fibre compared to methanol extracts (Al-Farsi and Lee, 2008). These results were in line with previously reported data that BP was a useful source of dietary fibre, making it potentially beneficial for individuals with high cholesterol levels (Niewiadomska et al., 2022; Tresina et al., 2022). Our current results confirm that T contains a high amount of dietary fibre and protein. Similarly, for dates (flesh + seeds), proximate analysis conducted in previous studies has consistently shown the presence of phenolics, dietary fibre, and antioxidant activity (Mia et al., 2020; Hinkaew et al., 2021).

Trace element deficiencies have been identified in diabetes, hypertension and obesity. Several elements have been repeatedly documented *in vitro* and *in vivo* to show insulin-like activity. In the current study, we found significant amounts of calcium (Ca) and magnesium (Mg) in the aqueous extracts of the materials tested. The presence of Ca in BP has been previously reported by Chinma and Igyor (2007). Ca plays key roles in muscle contraction and synaptic transmission. In the context of hypercalcaemia, an elevated level of Ca may also exert therapeutic effects. Our results showed that BP contained high amounts of magnesium, iron and zinc. This finding aligns with previously reported research by Nwofia et al. (2013), which suggested that BP may have the potential to be used as a remedy to alleviate malnourishment (Nwofia et al., 2013). Similarly, in AP, a high concentrations of Ca and Mg have also been reported (Assirey, 2015).

The qualitative analysis of BP, T, AP, and AS aqueous extracts showed that they contained fairly high levels of phenols and steroids. BP and AP extracts contained tannins, while alkaloids were present in the extracts of T and BP. These findings were consistent with previous research on BP, which identified reasonably high levels of glycosides, phenols, and tannins (Sindhu et al., 2013; Kadam et al., 2013). Kaempferol and quercetin have been shown to improve glucose uptake without adipogenic activity (Zhang et al., 2019). The current work found that phenolic compounds were present in AS. Previous studies indicated that date fruits contained significant amounts of phenolic acids, flavonoids, carotenoids, and steroids (Al-Jasass et al., 2015). Thus, it can be suggested that phenolic compounds may contribute to antioxidant capacity of the plants of interest. Other studies have also reported the presence of significant amounts of phenolics, flavonoids, and dietary fibre in date seeds, along with high antioxidant activity, such as radical-scavenging capacity and high reducing power (Akasha et al., 2012; Platat et al., 2014). Moreover, other authors conducted phytochemical analysis of AP and found that ajwa date was rich in polyphenols such as rutin, caffeic acid and catechin (Nehdi et al., 2010; Ragab et al., 2013).

Flavonoids and phenolics exhibit strong free radical scavenging potential, antimutagenic and signalling properties, as well as metal-chelating abilities. They were also found to exert a protective effect on human health (Vasco et al., 2008; Sarfraz, 2020). There is a strong relationship between plant phenolic contents and the antioxidant potential of these plants. In addition, a positive association between the antimutagenic and antioxidant potential of phenols has been reported (Singh et al., 2012a). HPLC is a reliable method for chemical profiling of plant extracts. Thus, a rapid, simple, and specific RP-HPLC fingerprinting was performed in the present study to quantify specific compounds, including piperine, curcumin, catechin, rutin, caffeic acid, gallic acid and quercetin (Springfield et al., 2005). Standards were selected based on therapeutic relevance, for example, quercetin shows antioxidant potential or can be used as an anti-inflammatory agent. It has also been suggested as a food supplement for managing hypertension problems (Boots et al., 2008; Sotnikova et al., 2013). Similarly, caffeic acid is useful in reducing acute immune and inflammatory responses. Moreover, catechin is able to activate multiple pathways, including the signalling of AMPK, PPARγ, Akt and ERK1/2. It possesses effective antioxidant and antitumour properties and has also shown anti-obesity effects in some studies (Huang et al., 2012; Luo et al., 2021; Lin and Lin-Shiau, 2006; Hursel and Westerterp-Plantenga, 2010). The natural compounds in the tested plant extracts may act by activating different pathways simultaneously. For example, curcumin (isolated from turmeric) was shown to activate the PPARγ

and AMPK pathways, and piperine and quercetin the PPARγ pathway (AlTamimi et al., 2021; Park et al., 2012; Khaliq et al., 2015). The biological properties of turmeric are largely attributed to the presence of the active ingredient curcumin (Yang et al., 2021). Fresh dates have been found to contain a significant amount of phenolic compounds (Al-Dashti et al., 2021). Date seeds in particular, have been identified as a rich source of phenolics and antioxidants. These bioactive phytoconstituents, such as flavonoids, have the potential to serve as a basis for the development of dietary supplements. Few alterations in known compounds, derived from natural sources, can change the biological activity of the native compound (Suresh et al., 2013).

The aqueous extracts of the studied plants have demonstrated a clear hypoglycaemic effect. However, the mean body weight did not differ significantly between the individual treatment groups. Similar effects have been reported in an earlier study for hypoglycaemic agents such as dried date powder (Nandhagopal et al., 2013). In the present work, a significant variation in glucose content was observed both in the normal control and hyperglycaemic rats. These differences could be attributed to the dietary fibre present in the test materials. Bioactive compounds present in the seeds have previously been found to exert beneficial effects, including increasing insulin secretion in rats with diabetes (Zhang et al., 2020). Moreover, increased dietary fibre intake was found to be effective in lowering serum glucose levels, thereby significantly reducing the risk of heart diseases (Tschiersch, 2012).

During diabetes, total protein concentration decreases, which may be due to reduced amino acid uptake, a consequent lower concentration of essential amino acids, and an elevated amino acid glycogenesis to CO_2 and H_2O (Johnson et al., 2012). The results of the present study revealed that the administration of the aqueous AS extract and its various combinations with other extracts did not significantly change the normal protein levels. Albumin levels are typically used to diagnose and treat liver and kidney disorders. In hyperglycaemia, reduced albumin levels may be due to massive liver cell necrosis, liver function deterioration, hepatic insulin resistance, and impaired oxidative phosphorylation of glycogen. However, in diabetic animals, hypoalbuminaemia is a common problem, usually attributed to the presence of diabetic nephropathy (Yao et al., 2018). In the current work, the aqueous extracts did not sufficiently control the level of albumin, implying a lack of albumin turnover effects.

Aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels are used to analyse and treat liver and heart disease. Elevated levels of transaminase can indicate myocardial infarction, muscular dystrophy or hepatic disease. ALT is a liver-specific enzyme. In our study, increased serum AST, ALT, and ALP activities indicated that hepatic dysfunction could be induced by diabetes. This is supported by previous findings that diabetic patients' livers can undergo necrosis. Thus, the increased serum AST and ALT activities suggested leakage of these enzymes from the liver cytosol into the bloodstream (Larcan et al., 1979; Navarro et al., 1993), indicating alloxan-induced hepatotoxicity. Some investigators observed altered activities of gluconeogenic enzymes like AST and ALT in alloxanised hyperglycaemic rats (He and Aoyama, 2003; Sarfraz et al., 2017). Meanwhile, in this study, a significant increase in serum AST and ALT activities was observed in the BP and AP-treated groups. However, the significant decrease in enzyme levels caused by different extract combinations could be attributed to a reduction in lipid peroxidation of the hepatocellular membrane due to alloxan induction. This could also result from increased regeneration of damaged hepatocytes. These findings are consistent with previous evidence showing that date seeds extract has a protective effect on the liver and can restore the normal liver function in rats against poisoning (Butler et al., 2022; Najafi, 2011). Similar conclusions were drawn by Madkor et al. (2011). It has been suggested that improvements in the diabetic diet can also play a role in protecting against liver abnormalities and other diseases (Hadrami and Al-Khayri, 2012). Type 2 diabetes patients often exhibit a higher incidence of liver abnormalities, which is linked to insulin resistance (Salih, 2013). Moreover, this information is in line with a previous study that observed a reduction in AST, ALT, and ALP levels in hyperglycaemic rats treated with a combination of date seeds, insulin, and date pits in fortified bread (El Fouhil et al., 2011; Halaby et al., 2014).

In diabetes, a close relationship exists between glucose homeostasis and renal damage. A significant proportion of people with diabetes suffer from kidney failure, and high plasma urea levels are indicative of renal dysfunction (Singh et al., 2012b; Almdal et al., 1990). Serum urea levels in diabetic rats are elevated due to reduced serum protein content. Increased levels of circulating amino acids and deamination result in the formation of large amounts of ammonia, which is eventually

converted to urea. During gluconeogenesis, the breakdown of amino acids results in increased urea production (Ganong, 2000). However, in the current study, serum uric acid level was within the normal range in diabetic control rats, and non-significant changes were observed in different treatment groups. The present study showed that serum urea levels normalised after administration of aqueous extracts of the test materials to hyperglycaemic rats. This was in line with earlier findings in garlic (Ziamajidia et al., 2017), onion (El-Demerdash et al., 2005) and Panax ginseng root extract (Sawiress, 2011). Other study have indicated that polyphenols and flavonoids present in plant extracts may be responsible for the decrease in serum urea levels and their potential nephroprotective effects (De Melo et al., 2018). Normal glomerular filtration is correlated with normal levels of creatinine in the serum. Creatinine is an important marker for renal dysfunction, and its level increases in acute and chronic renal insufficiency. In the present study, untreated diabetic rats showed an increase in serum creatinine levels, but a slight decrease was observed in the extract-treated groups. This could indicate a reversal of this dysfunction.

In this study, aqueous BP, AP and AS extracts administered to the hyperglycaemic rats demonstrated effective normalisation of the histoarchitecture of the liver and kidney. Particularly, the combination of BP + AS and the aqueous extracts of all tested materials showed liver protection, indicating strong synergistic activity and the potential to prolong the regenerative effect of glibenclamide. The histopathological examination revealed that the liver, pancreas and kidneys were the prime targets of alloxan-induced toxicity. These findings are consistent with previous reports where adverse effects of alloxan toxicity were prevented by administering different doses of herbal extracts (Alezandro et al., 2013; Marton et al., 2021). In addition, Marton et al. (2021) reported that a group of alloxan-poisoned rats showed vascular congestion, monocellular and interstitial haemorrhages, cell degeneration, and organ necrosis.

Conclusions

Overall, the findings of this study highlight the antidiabetic and antioxidant potential of black pepper, turmeric, ajwa date pulp, seed, and their mixtures in rats with alloxan-induced diabetes. These natural ingredients hold promise as complementary or alternative therapeutic options for managing diabetes and reducing its associated complications. However, further research is needed to elucidate the underlying mechanisms and determine the optimal dosage and duration of treatment to maximise its efficacy. Furthermore, clinical trials involving human subjects are necessary to validate the findings from animal studies and establish their relevance and safety in clinical settings.

Conflict of interest

The Authors declare that there is no conflict of interest.

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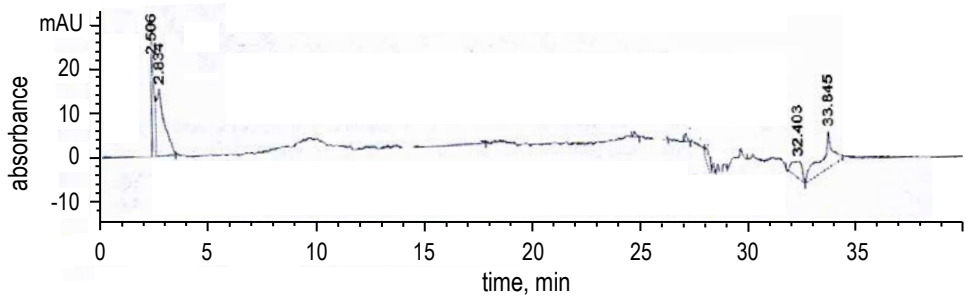
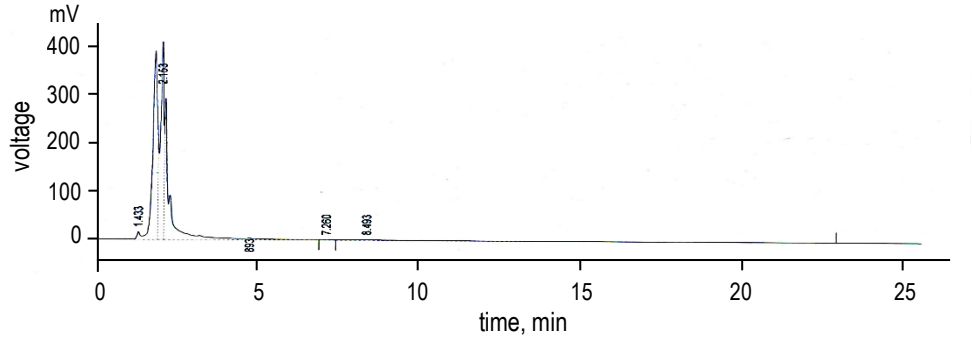
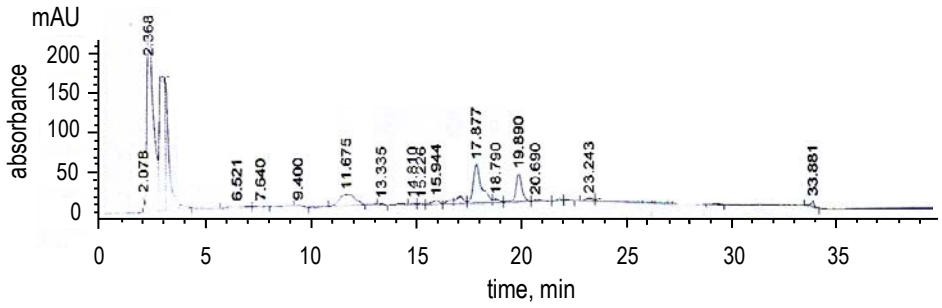
Supplementary material



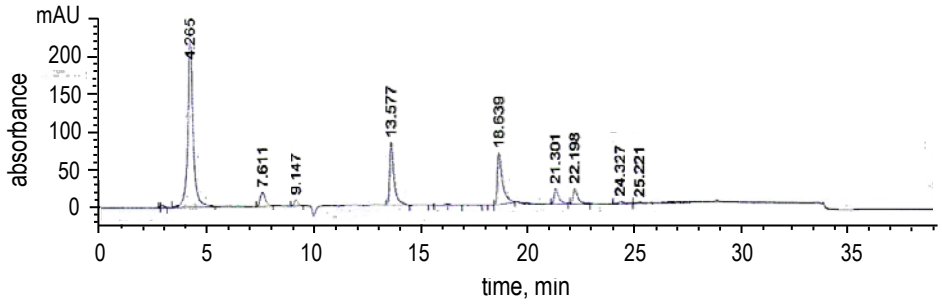
Appendix 1. High-performance liquid chromatography (HPLC) profile of black pepper (piperine)



Appendix 2. HPLC profile of turmeric (curcumin)



Appendix 3. HPLC profile of Ajwa pulp



Appendix 4. HPLC profile of Ajwa seeds