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Research Article

GC-MS Phytochemical Profiling, Antidiabetic, and Antioxidant Activities of *Khaya senegalensis* Stem Bark and *Azadirachta indica* Leaves Extracts in Rats

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This study was performed to evaluate phytochemical composition of *Khaya senegalensis* stem bark and *Azadirachta indica* leaf hydroethanolic (80%) extracts using GC-MS technique as a tentative identification method and screen for antioxidant and antidiabetic properties in Wistar rats. Diabetes was induced by intraperitoneal injection of alloxan (150 mg/kg·bw). Animals were divided into groups of six and treated by extracts (400 mg/kg·bw) for 28 days. The results compared with positive and negative control groups of animals. After treatment, blood samples were collected to determine the blood glucose level, lipid profile, liver and kidney function markers, and DPPH free radical scavenging activity was evaluated. Phytochemical investigations revealed that extracts were enriched with a wide range of secondary metabolites such as phenols, saponins, triterpenes, alkaloids, flavonoids, sterols, fatty acids, siloxane derivatives, and anthraquinones in diverse concentrations with reported antioxidant and antidiabetic properties. Biological screening results indicated that both extracts exhibited free-radical scavenging property in DPPH screening, and in that, *K. senegalensis* stem bark extract (91 ± 0.02%) showed greater reduction than *A. indica* leaf extract (55 ± 0.03%), with an IC_{50} of 0.023 ± 0.03 g/mL, which was lower than the reference drug propylgallate (0.077 ± 0.03 g/mL). Both the extracts remarkably reduced the blood glucose concentration in diabetic rats ($p < 0.05$). However, *A. indica* leaf extract showed greater reduction (52%) than *K. senegalensis* stem bark extract (37%). Similarly, the cholesterol, LDL, triglyceride, total protein, albumin, urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels decreased significantly ($p < 0.05$), in comparison to diabetic control animals. However, the concentrations of HDL slightly increased. Overall, both extracts showed significant antidiabetic and antioxidant potential in diabetic rats. As oxidative stress is associated with the hyperglycemia, the antioxidant activity displayed by the extracts will provide additional benefits in the antidiabetic therapy.

1. Introduction

Diabetes mellitus (DM) is among the most prevalent noncommunicable illnesses, and its influence on morbidity and death rates is continuously growing [1]. Unhealthy lifestyle, food habits, lack of exercise, obesity, and stress are considered as main factors responsible for growing cases of type 2 diabetes. Insulin deficiency and insulin resistance are the main causes of development of diabetic conditions which have certain effects on the carbohydrate, fats, and proteins metabolism and would significantly disturb water and electrolyte homeostasis [2]. Recent reports showed that the prevalence type 2 DM is common among Saudi population and is on the continuous rise. According to the recent information, the prevalence of DM in Saudi Arabia would be doubled, and half of the population may have DM by 2030, if increased at the current rate [3, 4].

Plant secondary metabolites play considerable roles in the treatment of various ailments in humans as therapeutic agents, either directly used to treat the diseases or providing potential scaffolds to be used as lead compounds in modern drug discovery. More than 500,000 low molecular weight natural compounds have been identified so far, which are potential for drug discovery [5]. Owing to the development of drug resistance, increasing cost of drug development, reduced rate of hits in modern drug discovery and higher adverse effects of synthetic drugs; the interest of medicinal scientists has been shifted towards the development of drugs from natural sources. The search for new secondary metabolites or synergistic combinations is extremely important as the spread of certain diseases rapidly growing and multidrug-resistant on the rise [6]. Alkaloids, flavonoids, phenolic compounds, polysaccharides, tannins, saponins, and steroids are the widely known biologically active compounds identified in plants. These compounds are well-recognized for their medicinal properties, including antidiabetic and antioxidant. Several of these phytocompounds are significantly being used for preventing and curing a wide range of illnesses and also exert defensive mechanisms against free radicals [7].

Khaya senegalensis (*K. senegalensis*) is a member of the family Meliaceae, found all over Saudi Arabia, where it is locally referred as Mahogany. It is a deciduous evergreen tree that grows to a 15–30 m height, with a diameter of up to 3 m and a clear bole of 8–16 m. *K. senegalensis* is among the popular plants in traditional medicine in African region used for treating various diseases [8]. The bark of the stem is used as bitter tonic to cure malaria, fever, mucous membrane diarrhea, and venereal disorders. Additionally, it is utilized as an anthelmintic and taeniacide. Several medicinal uses for the bark include aphrodisiac, blood tonic, and antimalarial [9, 10], as well as for wounds, diarrhea, and dysentery treatments [11]. In recent times, the plant has been studied for phytochemical investigation and screened for diverse biological properties including antidiabetic and antioxidant activities [12–15].

Azadirachta indica (*A. indica*) is a member of the Meliaceae family. In Saudi Arabia, it is referred as Neem. It is commonly used in folk medicine to treat various diseases including a range of bacterial and viral infections, bronchitis, skin conditions, septic sores, infected burns, hypertension, hypercholesteremic condition, diarrhea, fever, and as disinfectant [16–18]. Leaf and root decoctions are used to treat snake and scorpion stings, as well as intestinal spasms, respectively [19]. The leaf infusion is used to cure malaria, fever, and jaundice [20]. Additionally, the powdered leaves are combined with water and used to treat freckles on the face, increase hunger by reducing stomach gas production and eradicating intestinal worms [21]. The bioactive flavonoids were isolated from neem tree bark and were tested for acaricidal activity with a LD50 value less than 7.2 mM [22]. Apart from medicinal applications, the neem parts are also tested for its insecticidal property [23, 24]. Neem is one of the most popular medicinal plants and extensively used in Ayurvedic, Homeopathic, and Unani systems of medicine and is said to provide freedom from all the diseases. It has attracted considerable attention of modern system of medicine due to a vast array of biological properties. Every part of the neem plant has exhibited medicinal properties, and more than 300 bioactive structurally diverse compounds have been identified so far. Among the parts, leaf has demonstrated remarkable biological properties including antioxidant and antihyperglycemic activities [16, 25, 26].

Several studies indicated that the phytochemical composition of a plant species considerably varies depending on the region of the plant origin and climatic condition around. This has further indicated that abundance and composition of plant constituents remarkably depend on the geographical region where the plant has been cultivated. Therefore, the marked difference in the biological activities of a plant species collected from different regions is due to the difference in the phytochemical composition. The literature survey revealed that the selected plants have been studied extensively with respect to phytochemical and biological screenings; furthermore, the antidiabetic and antioxidant potential of both the plants has already been established by several studies, and there is scarcity of phytochemical composition data and concrete information on target biological activities of these plants grown in Saudi Arabia. The above observations necessitate the investigation of the selected plant species. Therefore, the current investigation was undertaken to evaluate the phytochemical composition of *K. senegalensis* stem bark and *A. indica* leaf collected from Jazan region, Saudi Arabia, by GC-MS analysis as a tentative identification method. The hydroethanolic extracts of both plants were screened for antidiabetic and antioxidant activities in Wistar rats.

2. Materials and Methods

2.1. Sample Collection and Extraction. *K. senegalensis* stems bark and *A. indica* leaves were collected from Jazan area of Saudi Arabia. Dr. Ramesh Moochikkal, a Botanist and curator of Jazan University herbarium; Department of Botany, College of Science, Jazan University identified and

authenticated the plant sample. Voucher specimen No JAZUH-1638 and JAZUH-1639 for *K. senegalensis* and *A. indica*, respectively, weredeposited in the herbarium of the Department for future reference. The samples were dried under shade until constant weight. The dried samples were reduced into small pieces, pulverized into powder, and weighed. The powdered samples were stored in air-tight containers in refrigerator until further use. The powdered stem bark and leaf samples (350 g each) were macerated in 750 mL of 80% ethanol, and extraction was made with occasional shaking for five days at room temperature. The mixture filtered through Whatman filter paper and filtrates from each sample was collected separately. Solvent from each filtrate was evaporated under pressure using rotary evaporator (Buchi, Switzerland) to brownish-black semi-solid extracts which were allowed to air-dry completely.

2.2. GC-MS Analysis of Extracts. GC-MS analysis has been used for tentative identification of the component of the extract. The dried plant extracts were reconstituted and diluted in methanol and analyzed by GC-MS system (Thermo Scientific) equipped with a trace ultra-GC, an AS 3000 auto-sampler, and an ISQ detector. The separation was accomplished with a TR 5MS column (Thermo Scientific) with dimensions of 30 m × 0.25 mm id and 0.25 mm film thickness. Helium was used as carrier gas at a flow rate of 1.2 mL/min (constant flow mode). Split-less mode sample injection was applied. The MS was operated in electron ionization (EI) mode with 0.6 scan periods throughout the mass range 60–900 AMU (min). The ion-source and transfer line temperatures were adjusted to 320°C and 350°C, respectively, using a 1 Kv electron multiplier voltage.

2.3. Identification of Phytochemical Components. Xcalibur software was used to analyze the mass spectra. Fragmentation patterns obtained for each compound in the extracts were compared to the standard mass spectral data available in the instrument database using the MAINLIB, NIST, and REPLIB built-in libraries. The percentages of components were calculated using peak areas as reference. Each component was identified by comparison with the structures in the computer library. The biological activities of compounds were obtained from Dr. Duke's Phytochemistry and Ethnobotanical Database.

2.4. Antidiabetic Activity. Thirty Wistar albino rats weighing between 150 and 250 g (age two months) were procured from Central Animal House of Jazan University, Saudi Arabia, kept in animal house facility of College of Pharmacy, Jazan University, in polypropylene cages (6 rats per cage) maintaining standard condition of 12 h cycle of light and darkness in an air-conditioned room. All the experimental rats allowed to clean water and pellet diet consisting of meat, wheat flour, carrots, and salt *ad-libitum*. One week prior to the testing, the rats were randomly grouped and acclimated to the normal laboratory conditions of temperature and humidity. All the procedures followed according to the Guide for Care and Use of Laboratory Animals [27]. This study was approved by the Ethics Committee of Jazan University, Saudi Arabia, before implementation.

2.4.1. Experimental Design. The animals were fasted overnight, weighed, and glucose levels were measured. Diabetes was induced into animals by administering single-dose intraperitoneal injection of alloxan monohydrate (Dop Chemical Company) dissolved in sterile normal saline (150 mg/kg·bw) [13]. To prevent hypoglycemic shock and death, the animals were given alloxan about six hours before being allowed to consume a 5% solution of glucose for the following 24 h. After 3 days of receiving alloxan, blood-glucose levels were recorded. Animals with fasting blood glucose concentration >200 mg/dl were considered to be diabetics [12]. For antidiabetic activity screening, the rats were randomly divided into 5 groups of six rats in each group. Group 1 was given distilled water (DW, 10 ml/kg·bw, no alloxan) and served as normal control group; Group 2 (diabetic control group) also given only DW (10 ml/kg·bw); Group 3, the positive control group received 5 mg/kg·bw glibenclamide, and Group 4 and 5 (antidiabetic test groups) received 400 mg/kg·bw of *K. senegalensis* stem bark and *A. indica* leaves extracts, respectively. According to earlier studies [28, 29], 5 mg/kg·bw dose of glibenclamide was used as the reference. The dosages of plant extracts used in this study were determined on the basis of the acute toxicity test results.

2.4.2. Blood Collection and Serum Preparation. After 28 days of the dosing period with 400 mg/kg·bw of extracts, the animals were fasted overnight and sacrificed under anesthesia on 29th day. Blood samples obtained from jugular vein in a sterile gel tube. Blood samples were collected in heparinized containers and centrifuged (IEG-CENTRA-4B centrifuge) at 5000 rpm at 4°C for 10 min for separating blood plasma. For the biochemical analysis, plasma were divided into aliquots and kept at −80°C until further use.

2.4.3. Biochemical Analysis. Diagnostic kits were utilized for analyzing the biochemical parameters including blood glucose level [30], lipid profile (cholesterol [31], low-density lipoprotein (LDL) [32], triglycerides [33], and high-density lipoprotein (HDL) [34], liver and kidney function markers including total protein [35], albumin [36], aspartate aminotransferase (AST), alanine aminotransferase (ALT) [37], creatinine [38], and urea [39] levels. The procedures described in the respective testing kits were followed.

2.5. Free Radical Scavenging Effect. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging tests were used to evaluate *in-vitro* antioxidant property of each extract. Methanol was used to dissolve the extract samples (1 mg/mL). The experiment was performed using 1, 5, 10, 20, 40, 60, 80, and 100 mg/mL concentrations of extract solutions. 96-well microtiter plates utilized, with 140 μL of 0.6×10^{-6} mol/L DPPH combined with 70 μL of the sample

in each well. The mixture was incubated in the dark for 30 min at room temperature, and optical density was recorded at 517 nm. The radical scavenging property of the extract was then calculated according to the formula mentioned in the literature [40]. The concentrations of the samples were plotted against the mean percentage of antioxidant activity (Sigma Plots R 2001, SPSS Science), and IC_{50} values were calculated. The IC_{50} obtained from the plots had a high coefficient of correlation ($R^2 = 0.998$), and the data presented as average standard errors of the mean (average SEM).

2.6. Acute Toxicity Evaluation. An acute oral toxicity study was performed according to the OECD No 425 Guideline's recommended upper limit (OECD/OCDE, 2008) [41]. Eighteen rats were fasted for 3 h and randomly divided into 3 groups with 6 animals in each group. Group 1 given distilled water (DW, 10 ml/kg·bw) and served as normal control group. The Group 2 and Group 3 were administered with a single dose of 2500 mg/kg·bw of *K. senegalensis* stem bark and *A. indica* leaves extracts, respectively. The rats were kept under observation for 14 days, with special attention during the first 6 h. The animals were observed for any physical and behavioral changes and mortality.

2.7. Statistical Analysis. The values are expressed as mean ± SEM. The measurement data between two groups (diabetic control group and test group) were compared through independent sample *t*-test. The results were considered statistically significant if p value less than 0.05 was observed.

3. Results

3.1. Antioxidant Activity. The findings of DPPH free radical scavenging activity revealed that *K. senegalensis* stem bark extract exhibited highest degree of activity (91 ± 0.02%) while *A. indica* leaves extract possessed a moderate activity (55 ± 0.03%). The inhibitory concentration (IC_{50}) of *K. senegalensis* was 0.023 ± 0.02 μg/mL. The results recorded for both the test extract were lower than that observed for standard propyl gallate (93 ± 0.01%, $IC_{50} = 0.077 \pm 0.01$ μg/mL). The free radical scavenging results have been presented in Table 1.

3.2. Effect of Extracts on Body Weights. The results of the study are presented in Figure 1. Treatment with both extracts and the standard drug used in this study have influence on the body weight of the diabetic rats when compared to diabetic control rats (untreated). Rats in diabetic groups receiving extracts showed an increase in body weight; however, the increase is less than the normal control animals. The control diabetic rats, which have not been treated with the plant extracts, showed a decrease in the body weight. Furthermore, it has been noticed that no significant change in the body weight of the diabetic rats receiving glibenclamide (standard drug) was observed.

3.3. Effect on Blood Glucose Level. The influence of hydroethanolic extracts from *K. senegalensis* stem bark and *A. indica* leaf was studied on diabetic rats (alloxan-induced). The animals were treated through administration of 400 mg/kg·bw for 28 days, and the glucose levels measured at 29th day. The results have been depicted in Figure 2. The results indicated that the glucose levels in treated rats were lowered by 37% and 52% by *K. senegalensis* stem bark and *A. indica* leaf extracts, respectively, in comparison to 68% reduction by the standard drug glibenclamide. On the other hand, diabetic control group rats receiving no treatment showed significantly higher glucose levels than normal control group rats. All the reductions were statistically significant ($p < 0.05$).

3.4. Effect on Lipid Profiles. Animals of all the experimental groups were evaluated for plasma lipid profile after receiving selected hydroethanolic extracts for 28 days, and the results compared with the normal control and diabetic control animals and those treated with glibenclamide. The results revealed that the cholesterol, triglyceride, and LDL levels remarkably increased in diabetic control group. However, increase in the cholesterol and LDL levels in the extract treated groups was minimal and less than diabetic control group. The cholesterol levels in the groups receiving *K. senegalensis* stem bark and *A. indica* leaf extracts were 84.20 ± 0.28 and 88.18 ± 1.86 mg/dl, respectively, in comparison to 127.47 ± 17.69 mg/dl recorded in diabetic control group ($p < 0.05$). The cholesterol level observed in normal control group was 76.70 ± 1.64 mg/dl. Similarly, the levels of triglyceride and LDL in *K. senegalensis* stem bark extracts (49.78 ± 8.52 and 27.76 ± 1.43 mg/dl, respectively) and *A. indica* leaves extract (47.39 ± 796 and 28.72 ± 2.39 mg/dl, respectively) were remarkably lower than observed in diabetic control group (94.31 ± 6.23 and 88.60 ± 11.58 mg/dl, respectively) whereas higher levels of HDL recorded in those animal samples received the extracts and standard drug treatments, when compared to diabetic untreated group. The results of lipid profile experiment obtained from extract treated and untreated rats have been summarized in Table 2.

3.5. Effect on Protein and Liver Function Markers. Following oral administration of *K. senegalensis* stem bark and *A. indica* leaf extracts (400 mg/kg·bw), total protein content along with liver function indicators (albumin, AST, and ALT) in the diabetic rats were evaluated. The results of the study are represented in Table 3. The total protein concentration was increased from 5.09 ± 0.14 g/dl (normal control group) to 7.10 ± 0.79 g/dl (diabetic control group) following induction of diabetes in rats. However, the protein content reduced to almost equal levels after 28 days treatment with hydroethanolic extracts from selected plants and standard drug glibenclamide ($p < 0.05$). Similar results were obtained on the plasma albumin levels, where the concentration of plasma albumin in diabetic control group was 2.11 ± 0.32 mg/dl, and after treatment with *K. senegalensis* stem bark, *A. indica* leaf extracts and glibenclamide were reduced to 1.50 ± 0.18, 1.46 ± 0.07, and 1.86 ± 0.39 mg/dl,

Table 1: DPPH free radical scavenging property results of 80% hydroethanolic extracts of *K. senegalensis* stem bark and *A. indica* leaves.

Plant/sample	Parts used for extraction	DPPH	
		RSA (%)	$IC_{50} \pm SD$ (μg/mL)
Khaya senegalensis	Stem bark	91 ± 0.02	0.023 ± 0.02
Azadirachta indica	Leaf	55 ± 0.03	ND
Propylgallate	Standard	93 ± 0.01	0.077 ± 0.01

*ND: not detected; RSA: radical scavenging activity.

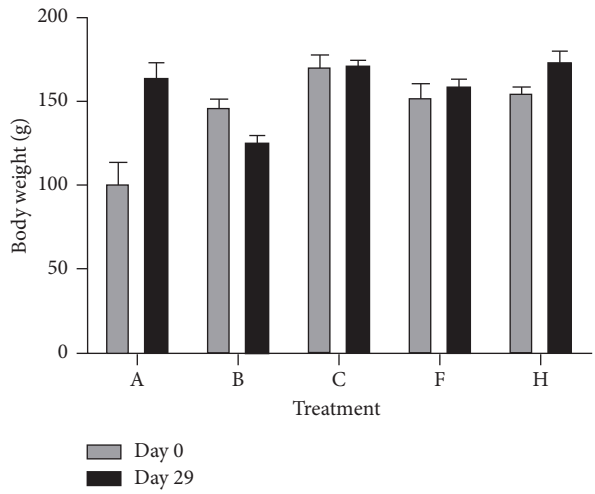


Figure 1: Effect of the *K. senegalensis* and *A. indica* hydroethanolic extracts on body weights of the diabetic rats. Results expressed as mean ± SD, $n = 3$ (with 95% confidence interval); A: normal control; B: diabetic control; C: glibenclamide; F: *K. senegalensis*; H: *A. indica*. Test period: 28 days.

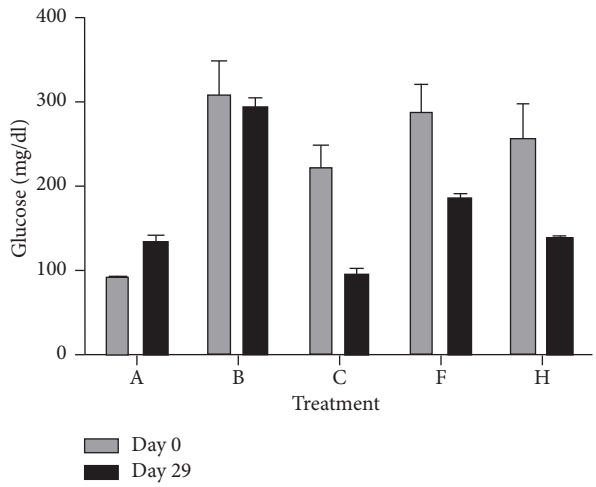


Figure 2: Effect of the *K. senegalensis* and *A. indica* hydroethanolic extracts on the blood glucose concentration of the diabetic rats. Results expressed as mean ± SD, $n = 3$ (with 95% confidence interval); A: normal control; B: diabetic control; C: glibenclamide; F: *K. senegalensis*; H: *A. indica*. Test period: 28 days.

respectively. Similar results were recorded as far as AST and ALT concentrations are concerned. Significantly elevated levels of AST and ALT were recorded in diabetic control group when compared to normal control group. However, the levels of AST and ALT were remarkably reduced following 28 days treatment with tested extracts and standard drug. The concentration of AST in the extract treated groups was between 147.08 ± 7.81 and 162.21 ± 9.88 U/I in comparison to 208.63 ± 14.52 and 123.34 ± 8.28 U/I in diabetic and normal control groups ($p < 0.05$). Similarly, the levels of ALT in treated animals were recorded between 44.74 ± 6.05 and 53.69 ± 4.77 U/I in comparison to 71.67 ± 3.78 and 38.71 ± 3.64 U/I in diabetic and normal control groups. However, the difference was statistically insignificant ($p > 0.05$).

3.6. Effect on Kidney Function Markers. Table 4 shows the influence of oral administration of *K. senegalensis* stem bark and *A. indica* leaf hydroethanolic extracts (400 mg/kg·bw) on the concentration of kidney function indicators including urea and creatinine in diabetic rats after 28 days treatment. The results of the study indicated that the urea and creatinine concentrations significantly increased in diabetic control group. However, treatment with either plant extracts or standard drug resulted in marked reduction in the levels of both the indicators. The urea levels in the groups receiving *K. senegalensis* stem bark and *A. indica* leaf extracts were 44.64 ± 12.48 and 54.37 ± 8.53 mg/dl in comparison to 83.67 ± 3.66 mg/dl recorded in diabetic control group ($p < 0.05$). The urea concentration observed in normal control group was 34.10 ± 5.40 mg/dl. Similarly, the level of creatinine in *K. senegalensis* stem bark and *A. indica* leaf extracts (0.66 ± 0.04 and 0.77 ± 0.03 mg/dl, respectively) was significantly lower than that observed in diabetic control animals (1.23 ± 0.26 mg/dl, $p < 0.05$). Similar results recorded with rats treated with glibenclamide. The statistical difference in the results between the treated groups and normal control groups was statistically insignificant ($p > 0.05$).

3.7. Acute Oral Toxicity Evaluation. The acute toxicity of *K. senegalensis* stem bark and *A. indica* leaf extracts was tested through oral administration of 2500 mg/kg·bw of each extract as single dose to the normal healthy animals selected for the study. The animals were kept on continuous observation for period of 14 days for any behavioral changes or death. However, no behavioral change or death in experimental animals was observed. Therefore, both the extracts were deemed to be safe up to a dose of 2500 mg/kg body weight when administered orally.

Table 2: Effect of oral administration of *K. senegalensis* stem bark and *A. indica* leaves hydroethanolic extracts on lipid profiles of diabetic rats.

Animal groups	Cholesterol levels (mg/dl)	Triglyceride levels (mg/dl)	HDL levels (mg/dl)	LDL levels (mg/dl)
Normal control (A)	76.70 ± 1.64	57.04 ± 11.99	38.13 ± 3.16	30.87 ± 8.26
Diabetic control (B)	127.47 ± 17.69	94.31 ± 6.23	31.74 ± 13.59	88.60 ± 11.58
Glibenclamide (C)	84.84 ± 3.55*	71.30 ± 7.83*	44.50 ± 3.18*	34.66 ± 5.80*
K. senegalensis extract (F)	84.20 ± 0.28*	49.78 ± 8.52*	37.71 ± 1.44*	27.76 ± 1.43*
A. indica extract (H)	88.18 ± 1.86*	47.39 ± 7.96*	47.18 ± 3.47*	28.72 ± 2.39*

*Statistically significant difference at $p < 0.05$, in comparison to diabetic control (group B). Values presented as mean ± SE; treatment period: 28 days.

Table 3: Effect of oral administration of *K. senegalensis* stem bark and *A. indica* leaves hydroethanolic extracts on protein and liver function markers of diabetic rats.

Animal groups	Total protein (mg/dl)	Albumin (mg/dl)	AST (U/I)	ALT (U/I)
Normal control (A)	5.09 ± 0.14	1.48 ± 0.05	123.34 ± 8.28	38.71 ± 3.64
Diabetic control (B)	7.10 ± 0.79	2.11 ± 0.32	208.63 ± 14.52	71.67 ± 3.78
Glibenclamide (C)	5.99 ± 0.19*	1.86 ± 0.39*	147.08 ± 7.81*	44.74 ± 6.05*
K. senegalensis extract (F)	5.08 ± 0.33*	1.50 ± 0.18*	151 ± 10.33*	53.69 ± 4.77*
A. indica extract (H)	5.43 ± 0.17*	1.46 ± 0.07*	162.21 ± 9.88*	49.36 ± 5.81*

*Statistically significant difference at $p < 0.05$, in comparison to diabetic control (group B). Values presented as mean ± SE; treatment period: 28 days.

Table 4: Influence of oral administration of *K. senegalensis* stem bark and *A. indica* leaf hydroethanolic extracts on kidney function markers in diabetic rats.

Animal groups	Urea (mg/dl)	Creatinine (mg/dl)
Normal control (A)	34.10 ± 5.40	0.63 ± 0.05
Diabetic control (B)	83.67 ± 3.66	1.23 ± 0.26
Glibenclamide (C)	28.20 ± 3.03*	0.69 ± 0.04*
K. senegalensis extract (F)	44.64 ± 12.48*	0.66 ± 0.04*
A. indica extract (H)	54.37 ± 8.53*	0.77 ± 0.03*

*Statistically significant difference at $p < 0.05$, in comparison to diabetic control (group B). Values presented as mean ± SE; treatment period: 28 days.

3.8. Phytochemical Screening. In this study, phytochemical screening of 80% ethanolic extracts of *K. senegalensis* stem bark and *A. indica* leaf was performed by GC-MS analysis, and the outcome has been summarized in Table 5. The outcome revealed that both the extracts contained a variety of chemical constituents including saponins, sterols, tannins, flavonoids, and triterpenes. Tannins were the major constituents in both the extracts; however, saponins and sterols represented other major phytochemicals in *K. senegalensis* stem bark extract. On the other hand, apart from tannins, triterpenes consisted of dominant phytochemical in *A. indica* leaf extract. Flavonoids detected in moderate amounts in both the extracts. The phytochemical metabolites such as coumarin and cyanogenic glycosides were not detected in this investigation.

In agreement with the studies reported in the literature, the GC-MS analysis performed in the current investigation indicated that the selected plant extracts are rich in phytochemical compounds possessing diverse biological activities including antioxidant and antidiabetic properties. Figure 3 represents the types of phytochemical constituents detected in GC-MS profiling of *K. senegalensis* stem bark and *A. indica* leaf extracts, and the individual components identified along with their retention time (RT), percent abundance, chemical structure, and biological properties are shown in Table 6. Two different types of fatty acid esters including hexadecanoic acid, ethyl ester (2.39%), and (E)-9-octadecenoic acid ethyl ester (0.79%) were identified in *K. senegalensis* bark extract. The major compounds with antioxidant and antidiabetic properties identified in *A. indica* leaf extract n-hexadecanoic acid (3.77%), methoxyphenyl-oxime (0.82%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(1.42%), phytol (7.4%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)-(3.28%), ethyl 9,12,15-octadecatrienoate (1.42%) and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl (1.41%), and β-sitosterol (0.69%). The ethyl esters identified in this analysis might have been prepared after extractive treatment with ethanol, and the ethyl portion was possibly supplied by the solvent to a fatty acid residue which is instead naturally present in the extract. Overall, the result indicated that *A. indica* leaves extract consisted of wider range of phytocompounds as compared to *K. senegalensis* stem bark extract.

4. Discussion

Despite considerable development of modern medicine for managing and treating diseases such as cancer, diabetes, hypertension, malaria etc., these diseases are continuously hitting the population globally with associated mortality. The plague of these diseases and discovering therapeutic agents that can be used for treating these diseases with little

Table 5: Phytochemical compounds detected in 80% hydroethanolic extracts from *K. senegalensis* stem bark and *A. indica* leaf.

Chemical compounds	*K.senegalensis* stem bark	*A. indica* leaves
Saponins	+++	++
Coumarins	—	—
Alkaloids	—	+
Anthraquinones	+	—
Tannins	+++	+++
Flavonoids	++	++
Sterols	+++	++
Triterpenes	++	+++
Cyanogenic glycosides	—	—

—Absent; + trace; ++ moderate; +++ high.

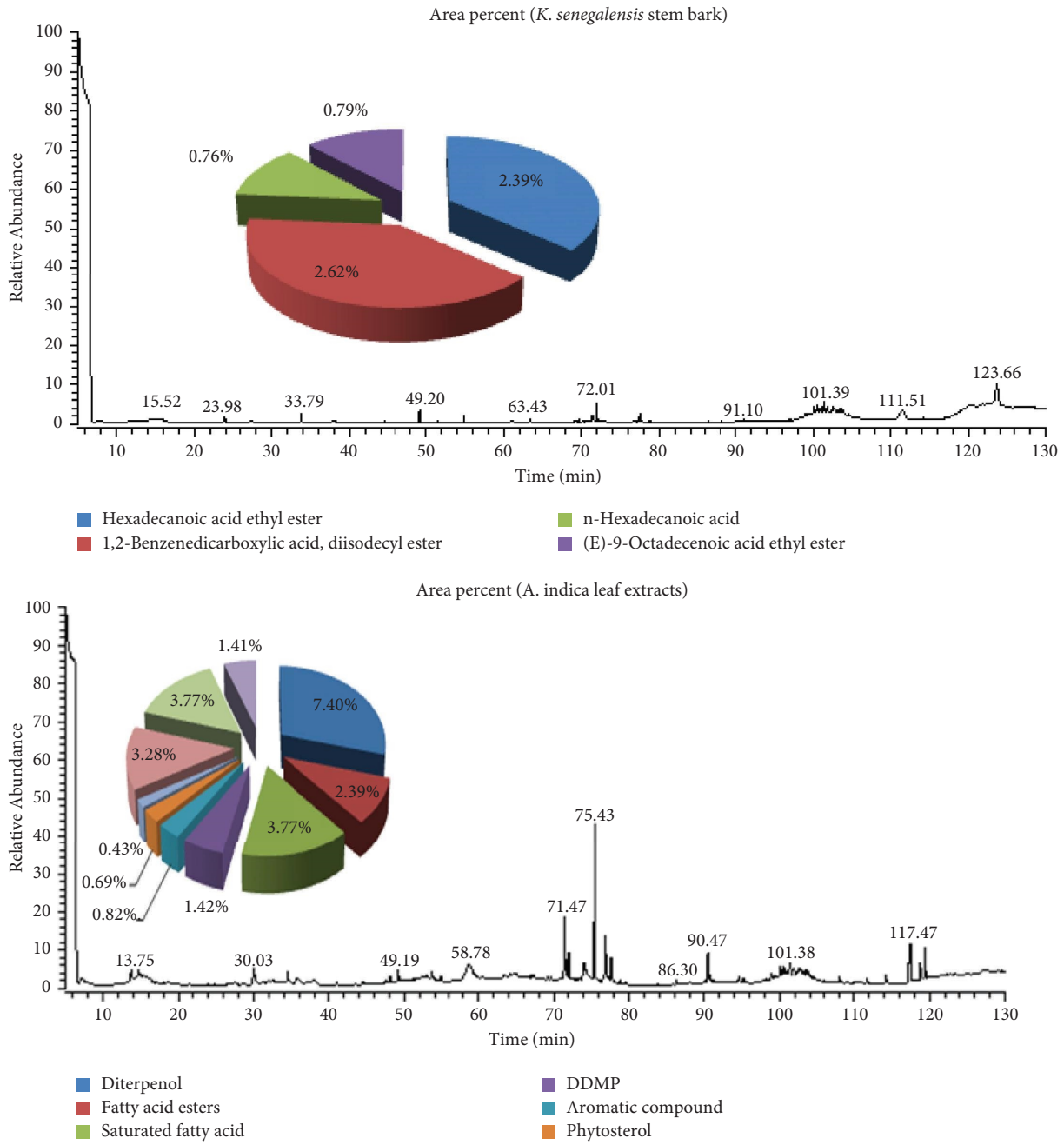


Figure 3: GC-MS profiling of *K. senegalensis* stem bark and *A. indica* leaves extracts.

Table 6: Phytochemical compounds identified in *K. senegalensis* stem bark and *A. indica* leaf extracts by GC-MS analyses.

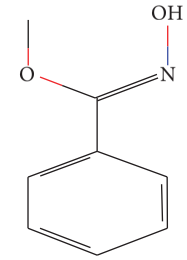
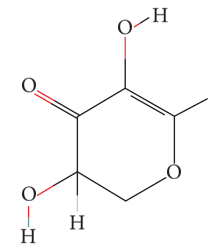
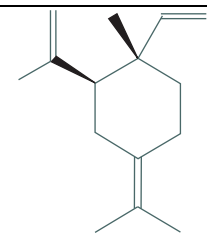
RT	Name of the compounds	Molecular formula	Molecular weight	% abundance* in *K. Senegalensis*	% abundance* in *A. Indica*	Structures and biological significance
13.75	Methoxy-phenyl-oxime	$C_8H_9NO_2$	151	—	0.82	 Antioxidant, antimicrobial [42]
30.02	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	—	1.42	 Antioxidant [43]
48.50	Elemene	$C_{15}H_{24}$	204	—	0.43	 Antioxidant, antibacterial [44]

Table 6: Continued.

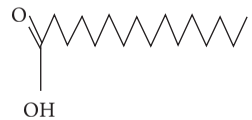
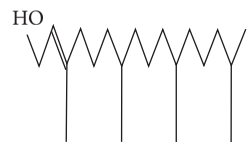
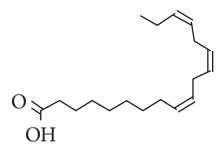
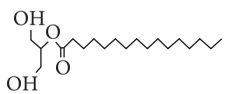
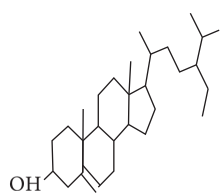
RT	Name of the compounds	Molecular formula	Molecular weight	% abundance* in *K. Senegalensis*	% abundance* in *A. Indica*	Structures and biological significance
71.40	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.76	3.77	 Antioxidant, hypocholesterolemic, 5-alphareductase activity [45, 46]
75.43	Phytol	$C_{20}H_{40}O$	296	—	7.40	 Antioxidant, antidiabetic, antimicrobial, anticancer, anti-inflammatory activities [45, 47]
76.89	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	—	3.28	 Antidiabetic, antidiabetic, anticancer, antimicrobial, antiatherosclerotic activities [45]
77.62	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306	—	1.42	Antioxidant, antiatherosclerotic, antidiabetic, antihypertensive, anticancer, hepatoprotective, antiarthritic activities [45, 48]

Table 6: Continued.

RT	Name of the compounds	Molecular formula	Molecular weight	% abundance* in *K. Senegalensis*	% abundance* in *A. Indica*	Structures and biological significance
90.47	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl	$C_{19}H_{38}O_4$	330	—	1.41	 Antioxidant, 17-beta-hydroxysteroid dehydrogenase inhibitor, arachidonic acid inhibitor, testosterone hydroxylase inhibitor, uric acid production inhibitor [45, 49]
114.23	β-Sitosterol	$C_{29}H_{50}O$	414	—	0.69	 Antioxidant, antidiabetic activities [47]

*% abundance represented the area percentage observed in the GC-MS chromatograms.

or no adverse effects is still a big challenge. Medicines from natural sources have played an important role in preventing and treating the human diseases since thousands of years. Several historical findings reported the medicinal properties of plants and their use for treating pathological conditions [50, 51]. According to WHO, herbal medicines play an important role in healthcare system, especially where modern medicines are not properly accessible to population [52]. Lack of accessibility to modern medicines, adverse effects, and high cost may be the main reasons behind the reliance of the population towards herbal medications. Indeed, this reliance is very obvious in the case of diabetes. However, a substantial proportion of plants used in traditional medicine have no or little scientific proof regarding their safety and efficacy. Therefore, it is imperative to conduct the phytochemical and biological screening of plants whose usage has been established in traditional medicine. The diverse biological properties exhibited by the plants are due to the presence of a variety bioactive phytochemical compounds including, alkaloids, glycosides, tannins, saponins, steroids, terpenoids, polysaccharides, and coumarins. However, the composition of these bioactive principles in different plant species is significantly different; even, the literature indicated that the phytochemical components of plant from same species collected from different geographic locations also vary. This difference leads to the variation in the nature and extent of biological property of the plant from same species collected from different locations. Therefore, the current study has evaluated antidiabetic and antioxidant properties of *K. senegalensis* stem bark and *A. indica* leaf 80% hydroethanolic extracts collected from Jazan region of Saudi Arabia on diabetic Wistar rats.

After induction of diabetes, the animals were observed for symptoms including weight loss, polydipsia, polyuria, and reduction in physical activities. In agreement with the literature, loss of weight among the diabetic control group of animals was noted, which is due to enhanced catabolic reactions leading to tissue protein breakdown and muscle wasting [53, 54]. The body weight of the animals administered with either selected plant extracts or standard drug was slightly increased after 28 days of treatment when compared with weight measured at first day of the study. However, it was significantly higher, in comparison to the diabetic control group ($p < 0.05$). The increase in the body weight was slightly greater in the animal received *A. indica* leaf extract in comparison to *K. senegalensis* stem bark extract (Figure 1). Increased body weight of the extract treated diabetic rats indicated that protein breakdown and muscle wasting were reduced. The treatment of diabetic rats by oral administration of 80% hydroethanolic extracts of *K. senegalensis* stem bark and *A. indica* leaf caused remarkable decrease in the blood glucose level in comparison to the diabetic control group ($p < 0.05$). The later showed greater antihyperglycemic action (52% reduction) in comparison to former extract (37% reduction). However, the antihyperglycemic activity exhibited by both the extracts was lower than the standard drug glibenclamide which showed 68% reduction in blood glucose concentration (Table 1). Furthermore, the glucose observed in both extract treated groups was slightly higher than the nondiabetic control group. Natural bioactive compounds produce antihyperglycemic activity through various mechanisms including enhanced β-cell proliferation, reduction in insulin resistance, and increased insulin secretion [55]. The reduction in glucose level after treatment with selected extracts may be achieved through these underlying mechanisms.

The biochemical parameters are associated with health conditions of the human being and are usually used as diagnostic tools in the clinical assessment of various diseases including diabetes. Parameters such as lipid profile, kidney and liver function indicators in normal, diabetic untreated, and diabetic animals treated with extracts and standards were evaluated in this experiment. There was remarkable decrease in the triglyceride, cholesterol, LDL levels in the animals treated with either *K. senegalensis* stem bark and *A. indica* leaf or standard drug glibenclamide when compared to diabetic control animals ($p < 0.05$). Slightly lower concentrations of cholesterol (84.20 ± 0.28 mg/dl) and LDL (27.76 ± 1.43 mg/dl) were observed in *K. senegalensis* stem bark extract treated group in comparison to *A. indica* leaf extract treated group (88.18 ± 1.86 and 28.72 ± 2.39 mg/dl, respectively). On the other hand, the later showed slightly better reduction in triglyceride level (49.78 ± 8.52 and 47.39 ± 7.96 mg/dl, respectively). No remarkable change in the HDL level was noticed. Indeed, the HDL level in the extract treated groups slightly increased as compared to the diabetic control group (Table 2). It is noteworthy that the lipid profiles of the diabetic rats after 28 days of treatment with *K. senegalensis* stem bark and *A. indica* leaf extracts became close to the normal control (nondiabetic) rats, indicating an effective treatment.

Concerning the liver and kidney function markers, 28 days treatment with *K. senegalensis* stem bark and *A. indica* leaf extracts significantly decreased the levels of total protein, albumin, urea, and creatinine in blood plasma, in comparison to diabetic control rats ($p < 0.05$). Increased levels of these parameters were observed following induction of diabetes with alloxan; however, similar to lipid profile, the levels of the above parameters came close to normal levels after 28 days of treatment with the tested extracts (Tables 3 and 4). The plasma concentrations of AST and ALT were also increased in alloxan-induced diabetic rats; however, remarkable reduction in the level of these parameters was recorded after 28 days treatment with extracts and standard drug ($p < 0.05$). Reduced levels of AST and ALT were recorded in the treated animals when compared with diabetic control animals (Table 3).

Overall, no serious toxic effect was seen after administration of *K. senegalensis* stem bark and *A. indica* leaf extracts at therapeutic dose (400 mg/kg·bw) for 28 days into the normal healthy and alloxan-induced diabetic rats because the levels of biochemical parameters including lipid profile, kidney, and liver function markers in the treated rats became close to their levels observed in normal rats. Evaluation of acute toxicity study was performed by administering high dosage of the extracts (2500 mg/kg body weight); however, only the behavioral changes and deaths among the test animals were observed. It was noteworthy that no behavioral

changes or deaths occurred in the animals receiving the high dosage of extracts, indicating no serious toxicity.

Individual phytochemicals or their combinations play a vital role in pharmacological effects. The hypolipidemic effect observed in this research could be due to wide range of phytochemical compounds such as tannins, terpenes, alkaloids, flavonoids, saponins, and sterols. All these secondary metabolites are known to include compounds that were found to reduce the plasma lipid level in animals, especially alkaloid, which is known for its insulinogenic effect on lipid metabolism and normalizes lipogenesis. It is also known that flavonoids reduce HMG-CoA reduction activity in the liver [56]. Phenolic compounds could also involve in the antihyperglycemic effect through inhibition of the alpha amylase enzyme activity. On the other hand, alkaloids could be involved in the inhibition of the enzyme alpha-glucosidase [57]. Terpenes may act as a glucagon secretion lowering and play an important role in the intake of physiologically important elements such as Cu^{2+} and Mg^{2+} for the functions of beta cells [58]. Furthermore, the antidiabetic effect of flavonoids is obvious as these compounds inhibit aldose reductase enzyme which catalyzes the conversion of glucose to sorbitol, which contributes to complications of diabetes [59]. Flavonoids and terpenes were found in both the tested extracts; these compounds were reported to exhibit antioxidant activity, and therefore, the antihyperglycemic potential of the extracts may be explained by elevation of insulin secretion through antioxidant activity [60]. Furthermore, the effect of plant extracts on diabetic rats may also be attributed to the possible enhancement of protein synthesis in the liver resulting in an increase in insulin secretion, and increase hepatic absorption of glucogenic amino acids [61]. This could also be due to decrease in proteolysis by activating the amino acids transamination enzymes [62].

Evidences indicating that hyperglycemia accelerates the generation of reactive free radicals causing oxidative stress are available in the literature. This further aggravates the development and progression of diabetic complications. Antioxidants influence signal transduction, cell proliferation, and immune responses due to their involvement in normal physiological processes. It has been found that oxidative stress has been associated with diabetes and studies indicated that antioxidants have shown potential benefits for cure and prevention of diabetes. Therefore, new treatment strategies for diabetes should involve the use of antioxidants as one of the therapeutic agents [63, 64]. With this view, both the selected extracts were screened for free radical scavenging activity in this experiment. Results of DPPH free radical scavenging activity of *K. senegalensis* stem bark and *A. indica* leaf extracts indicated that both the extract exhibited good potential. However, *K. senegalensis* stem bark extract exhibited greater activity (91%) than that produced by *A. indica* leaf extract (55%). The free radical scavenging activity produced by the former extract was comparable to the standard drug, propylgallate (93%). The observed antioxidant effect exerted by *K. Senegalensis* bark extract could be due to the identified fatty acids and their esters such as hexadecanoic acid, 9,12,15-octadecatrienoic acid, (Z,Z,Z)-, and ethyl 9,12,15-octadecatrienoate in GC MS analysis [45].

The antihyperglycemic activity of the tested extracts found in this investigation was in agreement with those reported by previous studies in the literature. In one study, Khosla et al. [65] reported antihyperglycemic property of *A. indica* leaf and oil extracts in rabbits against alloxan-induced diabetes. They concluded that the plant was capable in controlling the blood glucose concentration in diabetics and also helpful as preventive therapy and delayed the onset of the disease [65]. In similar study, aqueous leaf extract of *A. indica* was effective in controlling the blood glucose levels in diabetic rats and normalized the concentration of serum insulin, insulin signaling molecules, and lipid profile. The study concluded that the extract was effective in the management of type-2 DM through improvement in glucose utilization by skeletal muscles and insulin signaling molecules [66]. Kolawole et al. [14] reported antihyperglycemic effect of *K. senegalensis* stem bark aqueous extract in alloxan-induced diabetic rats; effects on glucose tolerance, body weight, and erythrocyte malonaldehyde concentration were also evaluated, and the effects comparable to glibenclamide were observed [14]. A similar effect on blood glucose concentration was observed in a study conducted in Nigeria. The study reported antihyperglycemic effect of the ethanolic extract of *K. senegalensis* stem bark along with reduction in alkaline phosphate and alanine transaminase levels in comparison to diabetic untreated animals [12]. The ethanol extract was also reported to exhibit free radical scavenging activity [15].

5. Conclusion

The current investigation was performed to evaluate the phytochemical profile of 80% hydroethanolic extracts of *K. senegalensis* stem bark and *A. indica* leaf extracts using GC-MS analysis and investigate the antidiabetic and antioxidant potential on alloxan-induced diabetic rats. The results indicated that both extracts significantly lowered the blood glucose levels and exhibited free radical scavenging activity. The activity observed was comparable to standard drugs. Lipid profile and plasma levels of kidney and liver function markers were also improved in diabetic rats following the treatment with selected extracts. The antihyperglycemic and antioxidant properties are attributed to various biologically active compounds detected in phytochemical screening of both extracts. The results also indicated that both extracts might be useful for treating diabetes mellitus. Moreover, it is well known that oxidative stress is one of the factors associated with the hyperglycemic condition; therefore, antioxidant activity of the extracts may offer additional benefits in antidiabetic therapy.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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