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Antihypertensive and Nephroprotective Potential of Endophytic *Aspergillus aculeatus*: Insight from *In-Vivo* and *In-Vitro* Mechanistic Studies

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Abstract

Background: Hypertension is a major cause for cardiovascular and renal diseases. The *Z. spina-christi* has a rich source of ethnomedicinal background and diverse bioactive compounds suggesting that its associated endophytic fungi may characterize a valuable source of novel therapeutic metabolite with potential applications in cardiovascular and renal diseases. **Objectives:** This research aims to investigate the antihypertensive and nephroprotective potential through endophytic fungus ZS1 isolated from *Ziziphus spina-christi in-vitro*, *in-vivo* and Renin-angiotensin converting enzyme inhibitory assays. **Methods:** The ZS1 strain was identified by Sanger sequencing and phylogenetic assay (NCBI, Mega-X). Ethyl acetate extract of the fungal culture was prepared and evaluated for antihypertensive activity in high-salt-induced hypertensive Wistar rats, with blood pressure, biochemical parameters, renin and ACE inhibition and kidney histopathology. Acute toxicity testing was conducted according to OECD guideline 425. **Results:** The fungal strain ZS1 was classified as *Aspergillus aculeatus* with GenBank Accession No. PQ192175. The extract showed dose-dependent reduction in systolic and diastolic blood pressure, with 200 mg/kg dose showing results comparable to those of captopril (30 mg/kg). *In-vitro* renin and ACE inhibition demonstrated dual inhibitory action, supporting modulation of RAS pathway as a potential mechanism of action. GC-MS analysis identified several bioactive compounds such as oleic acid, n-hexadecanoic acid, octadecanoic acid, and thymol, which are well known for their antihypertensive, anti-inflammatory,

and antioxidant properties. Histopathological analysis of renal tissues revealed significant restoration of normal architecture, particularly at higher dose, officiating a significant nephroprotective activity. **Conclusion:** These findings suggested that *Aspergillus aculeatus* represents a promising natural source of antihypertensive and nephroprotective agents. Further studies focusing on isolation of active compounds and elucidation of their molecular mechanisms are required to explore their therapeutic and pharmaceutical potential.

Keywords

Aspergillus aculeatus, Hypertension, Renin Inhibition, ACE Inhibition, GCMS Analysis

1. Introduction

Hypertension (high blood pressure) is a complex and progressive cardiovascular condition that significantly impacts global morbidity and death [1]. According to the World Health Organization (2021), over 1.28 billion adults worldwide are affected by hypertension, with a large number of people living in developing countries where access to adequate treatment and monitoring is limited [2]. Chronic hypertension is a primary cause for heart, kidney diseases and stroke [1]. However, several antihypertensive drugs were widely used like angiotensin converting enzyme (ACE) inhibitors, an angiotensin II receptors blockers, calcium channels blockers, and beta blockers. Their long term used is associated with harmful side effects, high cost, and patient non-compliance. These limitations highlight the need for alternative, safer, nontoxic, and cost effective pharmaceutical compounds [3].

Ziziphus spina-christi (Christ's thorn jujube), is a medicinal plant belonging to the family Rhamnaceae, widely distributed in the Middle East, Asia and North Africa where it has been used for centuries in traditional medicines. Leaves, fruit, bark and roots of this plant are used for the treatment of different diseases like diabetes, gastrointestinal diseases, skin and infectious diseases, inflammation and urinary tract infections. Its ethnopharmacological activities also are documented, as in the management of hypertension, edema, and kidney-related disorders. Indicating its significance to cardiovascular and renal health [4] [5].

Endophytic fungi that inhabit plant tissues are highly recognized for their ability to produce various bioactive metabolites, such as alkaloids, peptides, terpenoids, and phenolics, many of which exhibit cardiovascular protective properties [6]. These metabolites frequently enhance the pharmacological activities of their host plants and have demonstrated antioxidant, anti-inflammatory, and enzyme-inhibitory actions, specifically targeting enzymes crucial to blood pressure regulation, such as angiotensin-converting-enzyme (ACE) and renin [3]. One of the most important hormones is renin angiotensin aldosterone system (RAAS) to maintain the blood pressure stable. ACE facilitates the angiotensin I conversion to the vasoconstrictor angiotensin II, whereas renin initiates the first phase of the

cascade by converting angiotensinogen into angiotensin I [6]. The over activation of this system is associated with hypertension, vascular remodeling, and renal impairment. As a result, medications that inhibit ACE and renin have been widely used as the target in the treatment of clinical hypertension. Exploring natural bioactive compounds targeting the RAAS pathway has therefore become a priority in drug discovery [3].

Given the pharmacological potential of endophyte, the current study focuses on to identify endophytic fungi from *Z. spina-christi* and to assess their antihypertensive potential through *in-vitro*, *in-vivo* and mechanistic assays: *in-vivo*, to evaluate the effects of fungal extracts on systolic and diastolic blood pressure by high salt diet induced hypertensive rat model; *in vitro*, enzyme assays targeting ACE and renin were conducted to elucidate potential mechanisms of action at the molecular level. This study aims to provide new insights into the therapeutic potential of plant-associated fungi such as endophytic fungi, the alternative source for the development of novel, natural antihypertensive agents.

2. Materials and Methods

2.1. Plant Sample Collection

Mature leaves and stem of *Z. spina-christi* were procured from Dargai, District Malakand, Khyber Pakhtunkhwa, Pakistan. It is located at 34˚30′21″ N latitude and 71˚54′12″ E longitude. The Malakand average temperature is 22.51˚C, with an annual precipitation of approximately 133.32 mm and 147.32 days of rainfall.

2.2. Endophytic Fungi Isolation

Plant tissues were surface sterilized by immersion in 70% ethanol and in 2% sodium hypochlorite for 3 - 5 min, and then rinsed three times with sterile distilled water to remove sterilized agents. The tissues were divided into small segments (about 0.5 cm) and inoculated onto potato dextrose agar (PDA) plates added with chloramphenicol (50 mg/L) to inhibit bacterial contamination. The PDA media consisted of potato infusion (200g/L), dextrose (20 g/L) and agar (15 - 20 g/L). The inoculated Plates were incubated at 28˚C for a period of 7 to 14 days. Emerged fungal colonies were subcultured on fresh PDA plates to obtain pure isolates [7].

2.3. Phylogenetic Assessment

Molecular methods were utilized to identify the particular fungal isolate ZS1. DNA was extracted with the standard Qiagen DNA extraction spin technique with a DNeasy mini-DNA extraction kit. Sequencing was conducted by First Base Laboratory (Apical Scientific) in Malaysia. PCR amplicons were sequenced using Celemics BTSeqTM. The internal transcribed spacer (ITS) region of fungal isolate was amplified by utilizing universal ITS1 (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATAGC). Consensus sequences were analyzed with the NCBI Genbank database utilizing the BLASTn tool to identify closely related taxa [8]. Multiple sequence alignments were conducted with MEGA-X.

The phylogenetic tree was created by neighbor joining tree method [9]. The sequences were submitted to GenBank, to provide accession number. Bootstrap values of 70% or above were significant for clade support [10].

2.4. Preparation of Extract

For extract preparation the fungal endophytes pure colonies were cultured in Czapek media (10 g of glucose, 10 g of peptone, 5 g of MgSO4, and 5 g of KCl in one liter of distilled water) to produce biomass and cultural filtrate. The cultivated flasks were subsequently incubated in a shaking incubator for 15 days at 30˚C and 120 rotations per minute. The 15-day culture filtrate was added with an equal volume of ethyl acetate in a 250 ml flask, sealed, cultured for an additional 3 days, and subsequently vaporized at 40˚C by using a rotary evaporator [11].

2.5. Test Animal

Fifteen male specific-pathogen-free Wistar albino rats (180 - 200) were carried out from the veterinary research institute Peshawar. Rats were accommodated in polypropylene cages under conventional laboratory circumstances (12-hour light/dark cycle, 22 ± 2˚C, relative humidity 50% - 60%) with viable laboratory meals. The experiment on rats were approved by the animal ethical committee of Abdul Wali Khan University, Department of Botany, Mardan with reference number (No. AWKUM/BOT/2024/567), and followed to the rules established by the National Research Council [12].

2.6. Acute Toxicity Test

To evaluate the acute toxicity of an extract on Wistar rats weighing followed by Guideline 425 of the Organization for Economic Cooperation and Development (Guideline, 2001). If the rats exhibited significant behavioral alterations, including tremors, muscle stiffness, excessive salivation, reduced appetite, increased tear production, diarrhea, and mortality, over a period of 15 days, indicating signs of toxicity [13].

2.7. Induction of Hypertension

Hypertension was induced in rats by administering a high-salt diet including 8% NaCl (w/w) for a duration of 4 weeks [14]. Systolic and diastolic blood pressure were assessed weekly with the non-invasive tail-cuff technique (CODA™, Kent Scientific, USA). Rats exhibiting systolic blood pressure ≥ 140 mmHg after 4 weeks were classified as hypertensive and chosen for the experiment [15].

2.8. Experimental Design

Rats were divided into five groups (n = 3 per group), Group I (Normal control) given standard diet with 0.5% CMC. Group II (Hypertensive control) were given elevated salt diet with 0.5% CMC. Group III (Positive control) were given high-salt diet with captopril (30 mg/kg body weight). Group IV (Test group 1) with

high-salt with fungal extract (100 mg/kg body weight). Group V (Test group 2) were given high-salt diet with fungal extract (200 mg kg body weight). Oral treatments were administered for a period of 21 consecutive days [16].

2.9. Blood Pressure Assessment and Biochemical Parameters

A diastolic and systolic blood pressure was measured on day 21 using the tail-cuff method. At the end of the experiment, blood samples tests were collected under light anesthesia via retro-orbital puncture. Serum was extracted and evaluated for creatinine and urea level, using standard commercial kits (BioSystems, Spain) [17].

2.10. Renin Inhibition Assay

The renin inhibitory activity was evaluated with the fluorometric method [18]. Angiotensin I synthesis was assessed fluorometrically using excitation/emission wavelengths of 340/490 nm with a fluorescence microplate reader (BioTek, USA). The percentage of renin inhibition was calculated using the formula:

% Inhibition = (F control − F sample)/F control × 100

Where F control indicates the fluorescence intensity of the control and F sample signifies the fluorescence in the presence of fungal extracts. Captopril served as the positive control.

2.11. Angiotensin-Converting Enzyme (ACE) Inhibition

The ACE inhibitory activity was assessed following the method [19], with minor changes. Through the help of ethyl acetate the release hippuric acid was extracted, evaporated, and dissolved in distilled water, with absorbance measured at 228 nm using a UV-visible spectrophotometer (Shimadzu, Japan). The percentage of ACE inhibition was determined using the formula:

Percentage inhibition= (control A − sample A)/a control × 100

Control A denotes the absorbance of the control, while sample A represents the absorbance in the presence of the fungal extract.

2.12. Nephrohistology

Upon conclusion of the experimental period, rats were sacrificed under profound anesthesia, and both kidneys were removed and preserved in 10% of neutral buffered formalin for 48 hrs. Kidneys tissue sections (5 μm thick) were synthesized with the help of a rotary microtome and affixed on glass slides. The sections were stained with hematoxylin and eosin (H&E) for comprehensive histopathological analysis. Slides were analyzed using a light microscope by 20x and 40x magnifications [20].

2.13. Qualitative Phytochemical Assessment

Extract phytochemical analysis were conducted using a GC-MS chromatograph (Agilent 7890 GC paired with 5977B MSD) using a DB-5MS/HP-5MS capillary column. Compound identification was conducted through the comparison of electron ionization spectra with the NIST Mass Spectral Library using the MS

Search Program, requiring a match of 80% or greater [21].

2.14. Statistical Analysis

All the data were presented as mean ± standard error of mean (SEM). Statistical significance among groups was evaluated using one-way ANOVA, followed by Tukey's post hoc test, employing GraphPad Prism (v9.0). A p-value less than 0.05 was considered as statistically significant.

3. Results

3.1. Fungal Isolation from *Z. spina-christi*

From the sterilized leave of *Z. spina-christi* four fungal strains, specifically ZS1, ZS2, ZS3, and ZS4, were isolated. ZS1 demonstrated antihypertensive potential in comparison to other fungal isolates by *in-vitro* Renin and ACE inhibitory assay.

3.2. Phylogenetic Analysis of ZS1 Isolate

Phylogenetic analysis of ITS gene sequence obtained from Sanger sequencing indicated that the fungal isolate ZS1 clustered with *Aspergillus aculeatus*, deposited in the NCBI Genbank. The BLASTn analysis revealed 97% sequence identity, a query coverage of 59% and an E-value of 0.0, indicating a substantial alignment with the ZS1 strain. Phylogenetic tree was conducted utilizing the neighbor-joining (NJ) method, employing the Tamura-Nei model of nucleotide substitution, and examined across 21 nucleotide sequences which supported by a bootstrap value of 73% from 500 replications (**Figure 1**). The ITS gene sequence of strain ZS1 has been submitted to the NCBI GenBank with the accession number PQ192175.

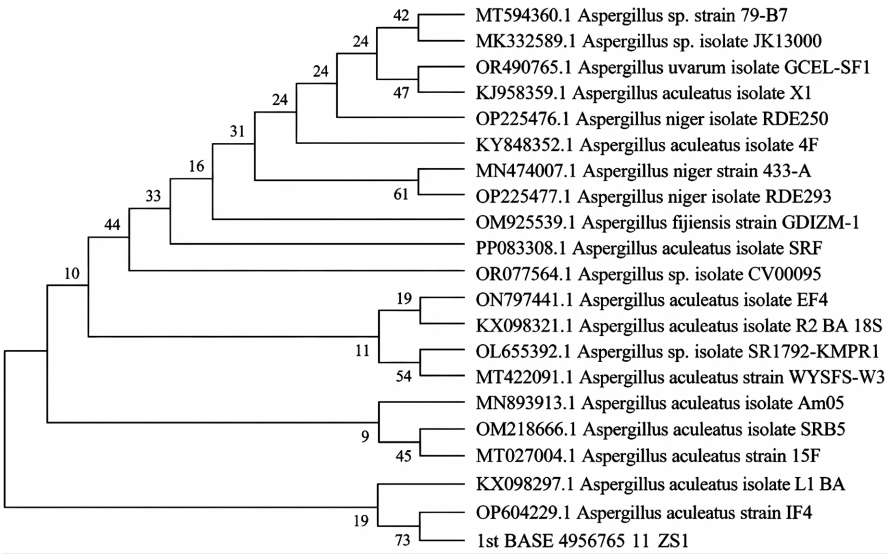

Figure 1. Phylogenetic tree of the isolate ZS1 constructed by the neighbor joining method on ITS sequence. Tamura-Nei model were used to compute the evolutionary distances assessed by 500 bootstrap replications. The ZS1 isolate clustered with *Aspergillus aculeatus* with 73% bootstrap support.

3.3. Acute Toxicity

The safety data revealed that the ZS1 extract was safe up to 400 mg/kg body weight concentration. There was no indication of behavioral changes, lethality and mortality in the rats under close observation, which considered a therapeutic advantage.

3.4. Blood Pressure Assessment

The hypertensive control group showed an elevation in both systolic and diastolic blood pressure compared to the normal control group. Captopril treatment significantly reduced blood pressure toward normal level, demonstrating the antihypertensive potential. Oral administration of *A. aculeatus* extract showed a dose dependent antihypertensive activity. The 200 mg/kg extract significantly lowered both systolic and diastolic blood pressure ($p < 0.05$) compared to hypertensive group (Figure 2(a)).

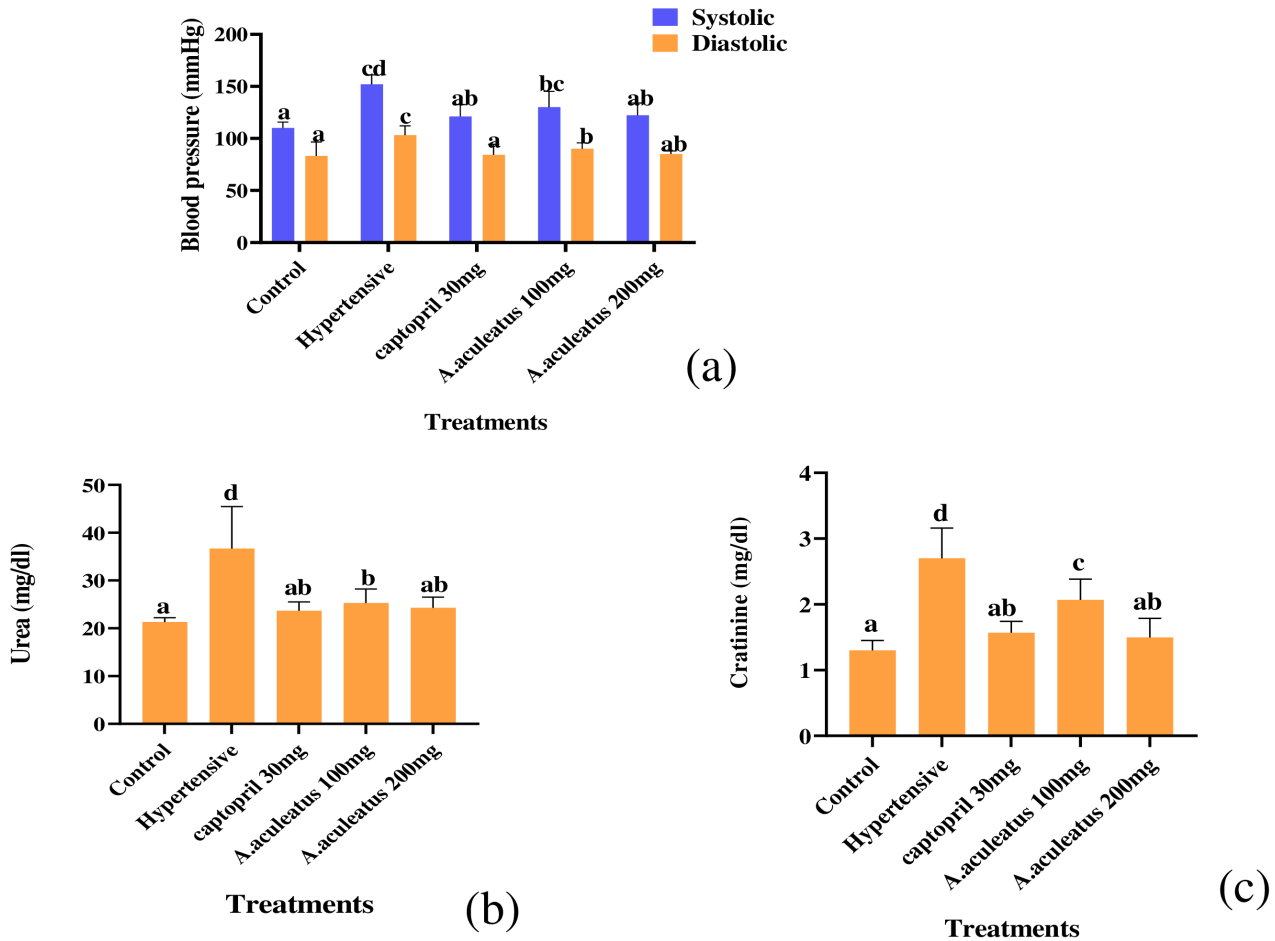

Figure 2. (a) Impact of *A. aculeatus* extract and captopril on systolic and diastolic blood pressure of high salt induced hypertensive rats. (b) Impact of *A. aculeatus* extract and captopril on serum urea level of high salt induced hypertensive rats. (c) Impact of *A. aculeatus* extract and captopril on creatinine level of high salt-induced hypertensive rats. Values are expressed as mean ± SEM (n = 6). Different letters (a, b, c, d) above bars indicate statistically significant differences between groups ($p < 0.05$, one-way ANOVA with Tukey's post hoc test).

3.5. Urea Level

The hypertensive group had a significant elevation in urea levels compared to the control group, indicating renal impairment associated with hypertension induced by a high-salt diet. Treatment with captopril (30 mg/kg) and *A. aculeatus* extract at 200 mg/kg significantly reduced urea level compared to hypertensive group. The 100 mg/kg dose of the extract reduced urea levels, but lesser extent than the higher dosage (Figure 2(b)).

3.6. Creatinine Level

The hypertensive control group demonstrated a significant elevation in serum creatinine levels relative to the normal control group, signifying renal dysfunction linked to hypertension. Captopril treatment (30 mg/kg) decreased serum creatinine, indicating a notable enhancement in renal function. Likewise, treatment of *A. aculeatus* extract at dosages of 100 mg/kg and 200 mg/kg reduced creatinine levels, exhibiting significant effects compared to hypertensive group (Figure 2(c)).

3.7. Renin and Angiotensin-Converting Enzyme (ACE) Inhibition

There was no inhibition of renin or ACE activity in the control group with hypertension. The treatment with captopril (30 mg/kg) inhibited renin (80.65%) and ACE (71.43%), thus demonstrating its potent effect on the RAS system. The extract of *A. aculeatus* had dose-dependent inhibitory activity. It inhibited renin (70.97%) and ACE (65.58%) at a dose of 100 mg/kg. Elevating the dosage to 200 mg/kg increased inhibition to 76.34% for renin and 70.78% for ACE, as shown in Table 1.

Table 1. Inhibition of renin and angiotensin converting enzyme by *A. aculeatus* extract.

Treatments	Renin (ng/mL/h) percentage inhibition	ACE (U/L) percentage inhibition
Hypertensive control	0.00 ± 0.20^{d}	0.00 ± 2.40^{c}
captopril 30 mg	80.64 ± 0.05^{a}	71.42 ± 1.45^{a}
A. aculeatus 100 mg	70.96 ± 0.05^{c}	65.58 ± 0.88^{b}
A. aculeatus 200 mg	76.34 ± 0.12^{b}	70.77 ± 0.57a

Note: Different letters (a, b, c, d) indicate statistically significant differences between groups ($p < 0.05$, one-way ANOVA with Tukey's post hoc test). (n = 6 per group).

3.8. Nephrohistology

In the nephrohistological analysis the control group (a) showed a normal kidney structure, glomerulus, bowman's capsules and regular renal tubules without inflammation. The Hypertensive group (b) displayed pronounced pathological alterations, including glomerular atrophy, tubular dilation, necrosis, interstitial edema, and inflammatory cell infiltration. Captopril-treated group (c) Exhibited partial recovery of renal morphology, with well-defined glomeruli and decreased

inflammatory infiltration, showed the renoprotective action of captopril. *A. aculeatus* 100 mg/kg group (d) Demonstrated mild recovery of renal histology, with less inflammatory infiltration and partial restitution of glomerular and tubular integrity. (e) *A. aculeatus* 200 mg/kg shown a nearby normal kidney morphology with improved glomeruli and minimal tubular or interstitial modifications, signifying a protective potential of fungal extract (Figure 3).

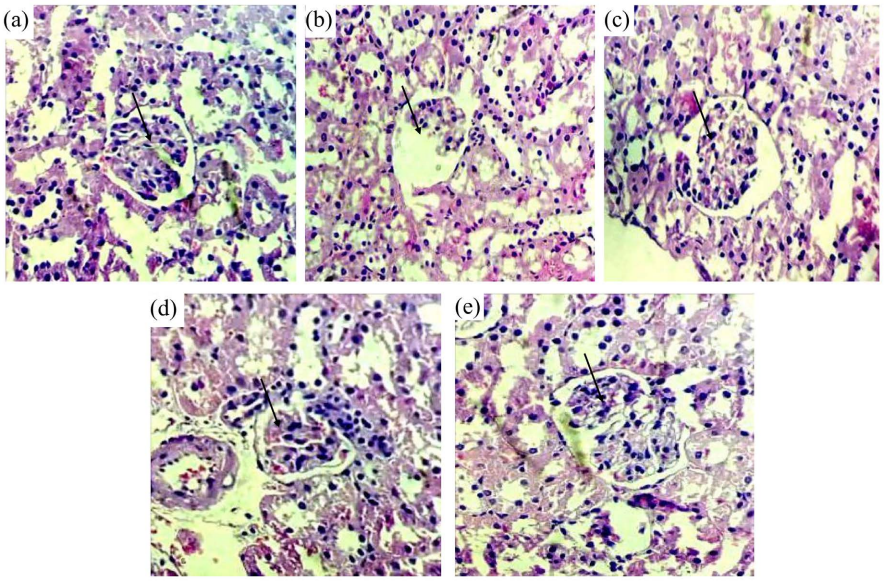

Figure 3. Effect of *A. aculeatus* and captopril on the histology of kidneys. (a) Control group; (b) Hypertensive group; (c) The Captopril-treated group (30 mg/kg); (d) The *A. aculeatus* treated group (100 mg/kg); (e) The *A. aculeatus* treated group (200 mg/kg). The arrows denote Glomerulus.

3.9. Phytochemical Analysis

Table 2. Compounds identified in the ethyl acetate extract of *A. aculeatus*.

S. No	Compound name	RT (min)	Area %	Molecular formula	MF
1	n-Hexadecanoic acid	17.426	8.12	$C_{16}H_{32}O_2$	256
2	Oleic acid	19.045	58.66	$C_{18}H_{34}O_2$	282
3	Octadecanoic acid	19.276	16.55	$C_{18}H_{36}O_2$	284
4	(9E,11E)-Octadecadienoic acid	19.376	4.67	$C_{18}H_{32}O$	280
5	4-[(Isopropyl)carbamoyl] benzoi	22.268	4.33	$C_{12}H_{15}NO_3$	207
6	Thymol	22.373	3.36	$C_{10}H_{14}O$	150
7	4-tert-Octylphenol	22.406	2.98	$C_{10}H_{14}O$	150
8	1,2-Bis(trimethylsilyl)benzene	22.210	1.32	$C_{12}H_{22}Si_2$	222

Note: RT: Retention time, MF: Molecular formula.

GC-MS analysis of ethyl acetate extract of endophytic fungal isolate ZS1 revealed a range of bioactive compounds as shown in Table 2 and Figure 4. The

peaks of fatty acid and their derivative such as oleic acid, n-hexadecanoic acid, and octadecanoic acid were observed in the chromatogram and were responsible for the major peak area together.

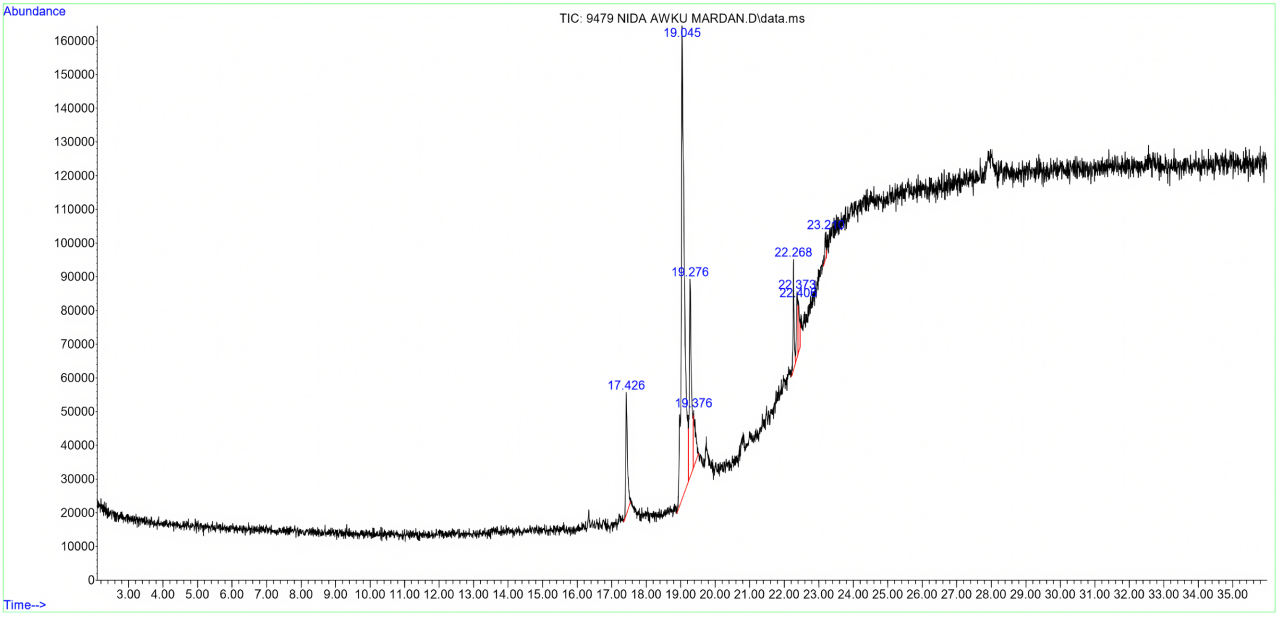

Figure 4. GC-MS chromatogram of *A. aculeatus* contained different compounds with different retention times and peak areas.

4. Discussion

The Species of the Genus *Ziziphus* have been utilized traditionally for conditions associated with hypertension, inflammation and metabolic disorders. Pharmacological studies demonstrated that *Ziziphus spina-christi* possess antioxidant, anti-inflammatory, antihyperglycemic, nephroprotective and cardioprotective activities which support many of its traditional medicinal applications. [5]. So the ethno pharmacological potential host plant suggest that its endophytes also have novel source of bioactive metabolites. Fungi also have antihypertensive activities, The peptides lle-phe and dipeptides significantly Inhibit ACE activity, demonstrated the therapeutic potential of fungal fermentation- derived bioactive peptides [22]. Similarly the Cordyceps militaris shows significant ACE-inhibitory activity. Cordycepin, one of the bioactive constituents of fungi which have antihypertensive potential capable of modulating the renin-angiotensin system [23].

The ZS1 strain identification closely clade with *Aspergillus aculeatus*, highlights the potential as source of bioactive compounds. The submission of the ZS1 ITS sequence in the NCBI GenBank (Accession No. PQ192175) confirms availability for future comparative studies and provides a taxonomic confirmation. It is particularly known for its capability to produce various secondary metabolites with antimicrobial, antioxidant and antihypertensive properties [3].

The high salt diet induced hypertension significantly elevated in both systolic and diastolic blood pressure in rats, studies indicating the role of dietary sodium

in the hypertension [24]. Administration of *A. aculeatus* extract at 200 mg/kg concentration significantly decrease the level of blood pressure.

A diet rich in sodium causes hypertension, resulting in renal impairment characterized by increased serum urea due to diminished glomerular filtration and changes in renal hemodynamics [24]. This study demonstrates that the significant rise in serum urea levels in the hypertensive rats supports the emergence of renal stress and possible nephrotoxicity associated with prolonged increased blood pressure. Treatment with *A. aculeatus* extract, at 200 mg/kg, significantly improved renal function, showed by a reduction in serum urea. The result was similar to that of synthetic captopril, indicating that the fungus extract possessed renoprotective and antihypertyensive properties. The potential of extract must be due to bioactive secondary metabolites found in the extract, including phenolics, alkaloids, and peptide-based compounds, which exhibit antioxidant, anti-inflammatory, and ACE-inhibitory characteristics [3].

Hypertension is also often found to be associated with renal dysfunction, as expressed through high serum creatinine levels due to compromised glomerular filtration rate (GFR) and increased oxidative stress in renal tissues [25]. The hypertensive control group in this research showed a significant increase in the values of creatinine, which shows the renal dysfunction caused by the model of high-salt diet. The control drug captopril and fungal extract at 200 mg.kg, successfully lowered creatinine levels, consistent with its recognized Reno protective effect via ACE inhibition, resulting in reduced intraglomerular pressure and improved renal hemodynamics. The results indicate that the bioactive compounds, identified by GC-MS like oleic acid, n-hexadecanoic acid and thymol, may play a vital role in correcting renal damage. These compounds are previously proven to be anti-inflammatory, antioxidant and protective to the kidneys [26].

The results demonstrate that the *A. aculeatus* extract has significant renin and ACE inhibitory actions, suggesting that its antihypertensive effects are partially mediated by the modulation of the renin-angiotensin system (RAS). Increased renin and ACE activities are essential in the development of hypertension, resulting in high level production of angiotensin II and aldosterone, which promote vasoconstriction, salt retention, and higher blood pressure [27]. The inhibition showed especially with the 200 mg/kg dosage producing effects similar to captopril, highlights the medicinal potential of *A. aculeatus*. The results support with other studies indicating that compounds from plants and fungi, play role as natural inhibitors of renin and ACE [3].

In Histological Analysis the Hypertensive group (b) Displayed pronounced pathological alterations, including glomerular atrophy, tubular dilution, necrosis, interstitial edema, and inflammatory cell infiltration, are characteristic of hypertensive nephropathy [28], which are due to prolonged high blood pressure and RAAS activation. Group Captopril-treated group (c) Exhibited partial recovery of renal morphology, with well-defined glomeruli and decreased inflammatory infiltration, setting the renoprotective action of captopril. The result indicated that the

ethyl acetate extract of *A. aculeatus* can modify the oxidative stress and improved the nephron structure.

In GC-MS different compounds were detected, among these, the most dominant one was oleic acid, which contributed around 58.66% to the total peak area. Oleic acid and omega-9 monounsaturated fatty acid, has been widely recognized for its cardioprotective, antihypertensive, and anti-inflammatory effects. It plays a role in lipid metabolism and vascular function by improving endothelial function and reducing oxidative stress [29] [30]. The n-hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid), are known to be saturated fatty acids with antimicrobial, anti-inflammatory, antidiabetic and antioxidant activity [31]. Thymol has anti-inflammatory, antioxidant and vasorelaxant activity which can have enhanced the antihypertensive efficiency of fungal extract [26].

5. Conclusion

This present research work revealed that *Aspergillus aculeatus*, isolated from *Ziziphus spina-christi*, exhibits potent antihypertensive activity against hypertensive rats induced with high salt. The extract lowered blood pressure, enhanced the structure of kidney tissue, and inhibited the actions of renin and ACE. GC-MS revealed identification of bioactive fatty acids and phenolic metabolites in the extract, which would be responsible for these pharmacological activities. Further studies will include bioassay-guided fractionation, structure elucidation of bioactive compounds, and preclinical assessment to determine the therapeutic potential of *A. aculeatus* as a new natural drug to manage hypertension.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

The experimental animal procedures were approved by the Department of Botany ethical committee, Abdul Wali Khan University Mardan.

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Author Contributions

Nida Gulal: Conceptualization, methodology, software, writing-original draft preparation. Husna Be-Misal: visualization, investigation, supervision, project administration. Altaf Hussain, and Bakhtbilanda: Formal analysis, investigation, resources, data curation. Muneeb Ur Rahman: validation and visualization, project administration of the study.

Conflicts of Interest

All authors confirm that there is no conflict of interest in this research.

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