Research Article

Assessment of Anti-Hypertensive Potential of *Colebrookea oppositifolia* by Angiotensin-Converting Enzyme Inhibition

Rukhsana Anwar[1*], Muhammad Usman Khalil[1] and Kanwal Ashiq[1,2]

[1]Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan; [2]Faculty of Pharmaceutical Sciences the Superior College, Superior University, Lahore, Pakistan.

Abstract | This study is aimed to evaluate the *in vivo* acute and sub-acute anti-hypertensive effect of methanolic extract of *Colebrookea oppositifolia* leaves. The sub-acute anti-hypertensive activity of a methanolic extract of *Colebrookea oppositifolia* 250 mg/kg were studied for 14 days in rats. Captopril (20 mg/kg) was given as a standard control. Non-invasive blood pressure (NIBP) monitoring technique was used to assess the blood pressure of rats. The frequency of hippuric acid synthesis from the hydrolysis of Hippuryl-L-Histidyl-L-Leucine was utilized to calculate angiotensin-converting enzyme (ACE) activity. The results showed that in comparison to hypertensive rats, the test doses of the extract significantly decreased systolic blood pressure (96.75±3.9 mmHg and 104±3.8 mmHg), diastolic blood pressure (73.12±5.2 mmHg and 65.37±8.2 mmHg), mean arterial blood pressure (81±4.1 mmHg and 78.25±6.7 mmHg), and heart rate (370±33.9 beats/min and 298.25±19.1 beats/min) respectively as compared to hypertensive rats. Sub-acute study results showed that the extract significantly decreased systolic blood pressure (97.33±3 mm Hg), diastolic blood pressure by (68.6±4.6 mmHg), mean arterial blood pressure (78.17±3.7 mmHg), and heart rate 335.8±27.5 beats/min as compared to hypertensive control rats. Furthermore, methanolic extract suppressed 45% ACE activity in hypertensive rats compared to the normal control group. According to the current findings, a methanolic extract of *Colebrookea oppositifolia* leaves decreases blood pressure via constraining the angiotensin-converting enzyme (ACE).

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***Correspondence** | Rukhsana Anwar, Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan; **Email:** rukhsana.pharmacy@pu.edu.pk
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Introduction

Medicinal plants are considered the cornerstone of traditional medicine (Singh, 2015). Since 4000-5000 B.C, medicinal plants have been used to treat various disorders (Danish *et al*., 2020). According to the World Health Organization (WHO), herbal medicines are used by approximately 80% of the world's population as these are effective, safe and cheap (Owolabi *et al*., 2007; Latif *et al*., 2019). Globally, cardiovascular diseases (CVDs) have been identified as the primary cause of morbidity and mortality,

accounting for 30% of fatalities, with around 40% of deaths happening in high-income nations and 28% occurring in low- and middle-income countries (Pallab *et al*., 2011; Ashiq and Ashiq, 2021). Furthermore, CVDs put extra economic burden compared to any other ailment. Hence, substantial studies are required to find effective and safe medicines to treat CVDs (Al-Shabanah *et al*., 1998). Among cardiovascular disorders (CVDs), hypertension is quite common. Hypertension is a chronic non-communicable disorder in which the blood pressure (B.P.) is elevated than its normal value (120/80 mmHg). Hypertension directly impacts one's quality of life (QoL) and is typically treated with either oral anti-hypertensive drugs or lifestyle changes such as exercise and dietary modifications (Beevers *et al*., 2011). It significantly raises the chance of developing additional serious disorders such as heart failure, coronary heart disease, angina pectoris, myocardial infarction, thump, renal failure, and blindness (Tabassum and Ahmad, 2011).

One of the mechanisms to lower blood pressure is controlled by renin angiotensin aldosterone system. Angiotensin converting enzyme play a key role in it by converting angiotensin I into angiotensin II. 1 Drugs not only control hypertension through ACE inhibition but also control cardiovascular diseases such as myocardial infarction (Shlipak, *et al*., 2001), CHF (Carson, 2000), as well as diabetic nephropathy (Hebert *et al*., 1999). Captopril currently available as antihypertensive drug exert effect by competitively inhibition of of ACE. Medicinal plants are the rich sources for developing new molecules.

Individuals from various areas of life use herbal therapies regularly. The use of herbal medications to treat cardiovascular disorders has increased in the ethnomedicinal system. Herbal therapies used for multiple heart illnesses such as congestive heart failure (CHF), hypertension, angina pectoris, arrhythmia, and vascular disease (Venkateshappa and Sreenath, 2013; Qasim *et al*., 2021). Herbal treatments have long been utilized in local communities worldwide to treat hypertension. This is an opportune time to evaluate and test the anti-hypertensive effects of medicinal herbs as these are safe and effective (Ashiq *et al*., 2019).

Colebrooke oppositifolia belongs to the *Lamiaceae* family and is found in India, Pakistan (Hussain *et al*., 2016), It is a valuable medicinal plant that can cure various ailments, including asthma, epilepsy, and CNS disorders. In a previous study, aqueous and methanolic extracts of leaves of *Colebrookea oppositifolia* were tested for cardioprotective activity against doxorubicin-induced cardiotoxicity. That is the only evidence available concerning the cardioprotective activity of *Colebrookea oppositifolia,* and the results of this study are quite preliminary (Pallab *et al*., 2011). According to a phytochemical study, it contains flavone glycosides and alkaloids, which are responsible for its main pharmacological effects (Madhavan *et al*., 2011). No research has been done to show that the *Colebrookea oppositifolia* has any anti-hypertensive activity. Therefore, this research aimed to examine the potential impact of *Colebrookea oppositifolia* inhypertension through ACE enzyme assay.

Materials and Methods

Animals

Male Wister rats weighing 120-150 g were obtained from Pakistan's Institute of Pharmaceutical Sciences (IPS-UVAS). For the current study, approval (No: AEC/PUCP/1059) was taken from the Animal Ethics Committee of the University College of Pharmacy, University of the Punjab, Lahore, Pakistan.

Plant extraction

Colebrook oppositifolia was collected in October. The plant authentication (specimen no: as GC.Herb. BOT.2973) was done by Prof. Dr. Zaheer-ud-Din Khan, a taxonomist at Government College University in Lahore. The leaves were carefully removed from the plant's twigs, rinsed and dried in the shade for two weeks. In total, 2 kg of leaves were pulverized. Approximately 500 g of powder was macerated for three days in 1 liter of methanol and covered with aluminum foil.

After three days, filtration of macerated material was performed to obtain the methanolic extract of leaves. The residues on the filter paper were macerated in methanol for another three days. Then, the obtained extract was dried by using a rotary evaporator. The dried methanolic extract yielded a total of 5.28% and was stored at 2-8 °C in properly labeled tarred glass vials.

Acute toxicity study

The objective was to find the LD_{50} of the methanolic

extract of the *C. oppositifolia* in rats. Swiss albino rats weighing 20-40 g were obtained from UVAS in Lahore, Pakistan. Rats were randomly assigned into six groups consisting of six rats in each group. Before administering the plant extract, their body weight was recorded. The death rate and any indicators of toxicity were monitored for 24 hours after a single dose of extract were administered orally to all groups. The maximum dose at which no mortality occurred was noted. Similarly, the dose at which all the animals died was found, and LD_{50} was determined using the given formula (Mushtaq *et al.*, 2017). The study design for the acute toxicity study is shown in Table 1.

$$LD_{50} = \sqrt{D_0 x\, D_{100}}$$

Where; D_0= Dose of 0% mortality; D_{100}= Dose of 100% mortality.

Induction of hypertension

In rats, hypertension was induced by giving them a 10% sucrose solution instead of water for 21 days. Each rat's body weight was recorded at the outset and throughout the trial. Computerized tail-cuff system (Power lab and Lab chart software by AD instruments) was used to record rats systolic blood pressure (SBP), mean arterial blood pressure (MAP), and heart rate (HR). Rats were trained for seven days with the tail cuff device to minimize noise readings. All animals were kept at 37°C prior to recording. Before the rat's treatment the BP measurement of each rat was carried out. Mean arterial and systolic blood pressure were used to compute diastolic blood pressure (DBP).

Table 1: *Study design for acute toxicity study (n=6 rats).*

No	Groups	Treatment
1	Group-I	Normal saline 10 mL/kg. P.O. as a particular dosage
2	Group-II	MECO 1000 mg/kg P.O. as a particular dosage
3	Group-III	MECO 2000 mg/kg P.O. as a particular dosage
4	Group-IV	MECO 3000 mg/kg P.O. as a particular dosage
5	Group-V	MECO 4000 mg/kg P.O. as a particular dosage
6	Group-VI	MECO 5000 mg/ kg P.O. as a particular dosage

MECO= Methanolic extract of Colebrookea oppositifolia.

Acute anti-hypertensive study

Rats were grouped into five groups (n=6) and were given treatment orally according to the following study design (Table 2).

Sub-acute anti-hypertensive research

The rats were habituated to the power lab, and baseline SBP, MAP, and H.R. measurements were taken using non-invasive blood pressure monitoring equipment (NIBP). The rats were given 10% glucose for 21 days to make them hypertensive. The rats were separated into the following groups (n=6) and treated orally according to the experimental design described in Table 3.

Table 2: *Study design for acute anti-hypertensive study (n=6 rats).*

No	Groups	Treatment for 21 days
1	Normal control	Normal rat chow diet and water *ad libitum*
2	Hypertensive control	10% glucose solution for 21 days
3	Standard control	10% glucose solution for 21 days + single dose of Captopril 20 mg/kg on 21st day
4	Experimental control-I	10% glucose solution for 21 days + single dose MECO 250 mg/kg on the 21st day
5	Experimental control-II	10% glucose solution for 21 days + a single quantity of MECO 500 mg/kg on the 21st day

Note: *On the same day, following treatment with conventional and experimental medications, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (H.R.), and blood sugar levels (BSL) were monitored after every 2 hours, i.e., at 0hr, 2hr, 4hr, 6hr, and 8hr.*

Table 3: *Study design for sub-acute anti-hypertensive study (n=6 rats).*

No	Groups	Treatment for 35 days
1	Normal control	Normal rat chow diet and water *ad libitum*
2	Hypertensive control	10% glucose solution for 21 days
3	Standard control	10% glucose solution for 21 days, and then a daily dose of Captopril 20 mg/kg was administered for further consecutive 15 days
4	Experimental control	10% glucose solution for 21 days, and then a daily dose of MECO 250 mg/kg was administered for further consecutive 15 days

On the 35th day, ACE inhibition activity, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (H.R.), and blood sugar levels (BSL) were measured.

In vivo angiotensin-converting enzyme inhibition activity

For preparation of rat lung homogenate in order to separate ACE. The rats of each sub-acute group were sacrificed under anesthesia and lungs were collected, blotted dry and weighed. Lungs were homogenized in cold 67Mm Trsima-HCl buffer (pH 7.8). Lungs were then homogenized by tissue homogenizer. Lung homogenate was centrifuged at 6000 rpm at 4°C for 15 min repeatedly until pelleting stooped. The final supernatant was stored at -80 °C until further useed for analysis (Abdulazeez and Kurfi, 2016).

With little modification, Cushman and Cheung's spectrophotometric technique (1971) was used to assess the angiotensin-converting enzyme (ACE) activity. The assay employed 0.2 mL of 3% (w/v) hypural-Lhistidyl-L-leucine (HHL), 0.1 M potassium phosphate buffer with 0.3 M NaCl (pH 8.3), and 0.05 mL enzyme. After 15 minutes of incubation, the reaction was stopped by adding 0.025 mL 01 N HCL and 2 mL ethyl acetate. Then, the upper layer was centrifuged for 2 minutes and kept in a dry bath at 100°C for 15 minutes. As a result of the reaction, hippuric acid was produced. Subsequently, 3 mL of distilled water was added to this reaction mixture, and a reading was taken by spectrophotometer at 228 nm. By enzymatic hydrolysis, 1 mole of hippuric acid was released per milligram of the sample per minute (Abdulazeez and Kurfi, 2016).

Statistical analysis

The data were statistically computed using Graph Pad Prism version 7.01 software. One-way ANOVA and Dunnett's tests were used to compare the means of groups. The results are expressed as Mean ± S.D. The value of the level of significance was kept as a $p < 0.05$.

Results and Discussion

Acute toxicity studies

Acute toxicity investigations revealed no mortalities occurred up to dosage 4000 mg for 24 hours. However, the animals treated with 5000 mg/kg P.O. plant extracts began to die within 2 hours of administration of the dosage, and all of the animals died within six hours of therapy. The LD_{50} values were calculated as 4472 milligram/ kilogram P.O.

$$D_0 = 4000$$
$$D_{100} = 5000$$
$$LD_{50} = \sqrt{D_0 x D_{100}}$$
$$LD_{50} = \sqrt{4000 x 5000} = 4472\ mg/Kg\ P.o$$

Acute antihypertensive study (*in-vivo*)

Effect of an acute dose of methanolic extract of *Colebrookea oppositifolia* leaves on SBP, DBP, MAP, H.R. and BSL in rats

The acute anti-hypertensive activity results showed that giving 10% glucose to hypertensive control group animals significantly ($p < 0.05$) increased their systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (H.R.), and blood sugar levels (BSL) associated to the normal control group. When compared to hypertensive control group animals, the animals treated with methanolic extract of *Colebrookea oppositifolia* leaves (both in doses of 250 and 500 mg/kg P.O.) as well as the animals treated with the regular drug of captopril (20 mg/kg P.O) had significantly ($p < 0.05$) reduced the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (H.R.). The results are presented in Tables 4, 5, 6, 7 and 8.

Table 4: *Effect of an acute dose of methanolic extract of Colebrookea oppositifolia leaves on systolic blood pressure in rats.*

Groups	Systolic blood pressure (mmHg) on day 21				
	0 hr	02 hr	04 hr	06 hr	08 hr
Normal control	97.2±3.2	--	--	--	--
Hypertensive control	127.2±3.9[a]	--	--	--	--
Standard control	127.2±2.8	117.2±3.5[b]	111.0±1.8[b]	106.5±5.5[b]	91.5±4.5[b]
Experimental control-I	125.0±4.6	119.2±2.7[b]	112.7±3.2[b]	107.7±3.7[b]	96.7±3.9[b]
Experimental control-II	127.2±3.9	119.5±2.5[b]	112.5±2.5[b]	109.0±2.2[b]	104.0±3.8[b]

One-way ANOVA was used in the analysis, monitored by Dunnett's test. Values are articulated as Mean ± S.D., (n=4). Sign [a] ($p < 0.05$) indicates a significant increase in systolic blood pressure by administering 10% glucose as related to control, while sign [b] ($p < 0.05$) indicates a significant reduction in systolic blood pressure of treated groups as compared to hypertensive rats.

Table 5: *Effect of an acute dose of methanolic extract of Colebrookea oppositifolia leaves on diastolic blood pressure in rats.*

Groups	Diastolic blood pressure (mmHg) on day 21				
	0 hr	02 hr	04 hr	06 hr	08 hr
Normal control	68.2±4.4	--	--	--	--
Hypertensive control	97.6±5.1[a]	--	--	--	--
Standard control	95.7±8.4	79.0±3.4[b]	84.0±5.2[b]	85.5±4.3[b]	73.8±6.4[b]
Experimental control-I	97.6±5.2	80.3±5.2[b]	81.2±2.5[b]	77.3±4.3[b]	73.1±5.2[b]
Experimental control-II	88.2±3.1	74.3±6[b]	66.7±5.2[b]	56.8±12.1[b]	65.3±8.2[b]

The analysis was carried out using one-way ANOVA and Dunnett's test. The standards are given as Mean SD (n=4). Sign [a] ($p < 0.05$) indicates a significant increase in diastolic blood pressure by administering 10% glucose as compared to control, while sign [b] ($p < 0.05$) indicates a significant decrease in diastolic blood pressure by treated groups as compared to hypertensive rats.

Table 6: *Effect of an acute dose of methanolic extract of Colebrookea oppositifolia leaves on mean arterial blood pressure in rats.*

Groups	Mean arterial blood pressure (mmHg) on day-21				
	0 hr	02 hr	04 hr	06 hr	08 hr
Normal control	82.2±6.3	--	--	--	--
Hypertensive control	106.7±4.1[a]	--	--	--	--
Standard control	106.2±6.2	91.7±3.1[b]	93.0±3.7[b]	92.5±4.7[b]	79.7±5.8[b]
Experimental control-I	106.7±4.1	93.6±3.2[b]	91.7±1.9[b]	87.5±3.3[b]	81.0±4.1[b]
Experimental control-II	101.2±2.9	89.3±3.4[b]	82.0±3.2[b]	74.2±7.7[b]	78.2±6.7[b]

The analysis was carried out using one-way ANOVA and Dunnett's test. The standards are given as Mean SD (n=4). Sign a (p=0.05) shows a substantial rise in mean arterial blood pressure by providing 10% glucose compared to the control group, but sign b (p=0.05) indicates a significant drop in mean arterial blood pressure of treatment groups compared to hypertensive rats.

Table 7: *Effect of an acute dose of methanolic extract of Colebrookea oppositifolia leaves on heart rate.*

Groups	Heart rate (beats/min) on day 21				
	0 hr	02 hr	04 hr	06 hr	08 hr
Normal control	367.5±28.6	--	--	--	--
Hypertensive control	427.5±62.1[a]	--	--	--	--
Standard control	395.5±27.3	346.7±18.5[b]	327.7±11.4[b]	322.2±12.6[b]	310.7±20.8[b]
Experimental control-I	427.5±62.1	377.5±46.5[b]	419.2±57[b]	402.5±55[b]	370±33.9±4.1[b]
Experimental control-II	358.2±6.4	352.2±23.5[b]	332.0±68.4[b]	328.2±44.8[b]	298.2±19.1[b]

The analysis was carried out using one-way ANOVA and Dunnett's test. The values are given as Mean SD (n=4). Sign [a] ($p < 0.05$) indicates a significant increase in mean heart rate by administering 10% glucose as associated with controlling, while sign [b] ($p < 0.05$) indicates a significant decrease in heart rate of treated groups as compared to hypertensive rats.

Table 8: *Effect of an acute dose of methanolic extract of Colebrookea oppositifolia leaves on blood sugar levels in rats.*

Groups	Blood sugar (mg/dl) on day-21				
	0 hr	02 hr	04 hr	06 hr	08 hr
Normal control	102.5±11.4	--	--	--	--
Hypertensive control	326.2±63.2[a]	--	--	--	--
Standard control	255.0±62.2	254.0±60.9[b]	250.0±62.5[b]	247.5±61.1[b]	248.5±61.2[b]
Experimental control-I	241.5±52.1	238.5±53.4[b]	233.2±52.4[b]	232.2±52.6[b]	229.2±53.7[b]
Experimental control-II	326.2±63.2	324.0±65.1[b]	322.2±65.0[b]	318.5±64.6[b]	312.0±61.8[b]

The analysis was carried out using one-way ANOVA and Dunnett's test. The values are given as Mean SD (n=4). Sign a (p=0.05) indicates a substantial rise in blood glucose level by delivering 10% glucose vs. control, whereas sign b (p=0.05) indicates a significant drop in blood glucose level in treated groups versus hypertensive rats.

Links Researchers

Subacute anti-hypertensive study (in-vivo)

Subacute methanolic extract of *Colebrookea oppositifolia* leaves a significant effect on rats' SBP, DBP, MAP, H.R., and BSL. When associated with the usual control group, the hypertensive control group experienced a substantial ($p < 0.05$) increase in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (H.R.), and blood sugar levels (BSL). The animals in the standard and experimental control groups exhibited trivial ($p < 0.05$) decreases in SBP, DBP, MAP, H.R., and BSL compared to the hypertensive control group. Numerical values for all of the parameters are shown in Table 9.

Effects of Colebrookea oppositifolia extract on ACE activity in hypertensive rats

In-vivo ACE inhibition activity (µmol/min) was performed to evaluate the effect of methanolic extract of *Colebrookea oppositifolia* and standard (Captopril) in hypertensive rats. The percentage inhibition for methanolic extract and standard was recorded as 45.13% and 60%, respectively, as shown in Table 10.

Colebrooke oppositifolia is widely used as a traditional herbal medication for hypertension and stroke-like conditions (Viswanatha *et al*., 2021). To test the anti-hypertensive efficacy, albino rats were given a methanolic extract of *Colebrookea oppositifolia*. Administration of 10% glucose will significantly increase rats' systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, heart rate, and blood sugar level ($p < 0.05$) compared to the control group.

In acute study, MECO 250 mg/kg and MECO 500 mg/kg and standard successfully reduced the systolic blood pressure ($p < 0.05$) by up to 23% and 18%, and 28%, respectively as compared to hypertensive rats. MECO 250 mg/kg MECO 500 mg/kg and Captopril substantially reduced diastolic blood pressure ($p < 0.05$) by 25%, 26%, and 23%, respectively. Reduction in mean arterial blood pressure by MECO 250 (24%), MECO 500 (23%), and captopril (25%), are almost similar which means that the mechanism in all these cases may be same.

MECO 250 mg/kg was used to study the sub-acute effects on SBP, DBP, MAP, H.R., and BSL. Compared to hypertensive rats, treated animals exhibited a reduction 23% systolic blood pressure, 18% diastolic blood pressure, 20% mean arterial blood pressure, and 20% H.R. as compared to the hypertensive rat group. Methanolic extract of *Colebrookea* inhibited ACE activity considerably (3.9±0.2 µmol/ min) against hypertensive rats (7.2±0.3 µmol/ min). Significant ACE inhibition is comparable with positive control group.

Table 9: *Effect of sub-acute doses of methanolic extract of Colebrookea oppositifolia leaves on SBP, DBP, MAP, H.R. and BSL in rats.*

Groups	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	HR (Beats/min)	BSL (mg/dl)
Normal control	96.5±3.6	72.3±8.2	80.3±6.0	354.3±32.4	88.3±9.2
Hypertensive	126±4.6[a]	83.5±6.3[a]	97.3±4.7[a]	421.8±51.5[a]	253.0±48.5[a]
Standard	95.0±3.8[b]	71.0±4.7[b]	79.0±4.0[b]	309.3±23.8[b]	258.8±39.6[b]
MECO 250mg/Kg	97.3±3.0[b]	68.6±4.6[b]	78.1±3.7[b]	335.8±27.5[b]	271.2±38.6[b]

SBP, Systolic Blood pressure; DBP, Diastolic blood Pressure; MAP, Mean Arterial Pressure; HR, Heart Rate; BSL, Blood Sugar Level. The analysis was carried out using one-way ANOVA and Dunnett's test. The values are given as Mean SD (n=4). Sign [a] ($p < 0.05$) indicates a significant increase in SBP, DBP, MAP, H.R. and BSL by administering 10% glucose as compared to control, while sign [b] ($p < 0.05$) indicates a significant decrease in SBP, DBP, MAP, H.R. and BSL in treated groups as compared to hypertensive rats.

Table 9: *In-vivo effects of Colebrookea oppositifolia extracts on ACE activity in hypertensive rats.*

Groups	ACE activity (µmol min^{-1})			
	Replicate-1	Replicate-2	Replicate-3	Mean ± SD
Normal control	7.6	6.8	7.1	7.2 ± 0.3
Mrthanolic extract 250 mg/Kg	4.2	3.7	3.8	3.9 ± 0.[2]b
Standard control (Captopril 20 mg/ Kg)	2.6	3.1	2.8	2.8 ± 0.2[b]

One-way ANOVA and Dunnett's test were used in the analysis. The outcomes are given as Mean SD (n=3). The probability value is set at b = 0.05. The suppression percentages for methanolic extract and standard are 45.1 and 60%, respectively.

Many studies have proved that phytochemicals like tannins and flavonoids are beneficial in reducing hypertension (Cienfuegos-Jovellanos *et al*., 2009). *Colebrooke oppositifolia* is a quite enriched source of many phytochemicals (Yadav, 2019). The plant contains various chemical classes, including flavonoids, tannins, polyphenols, alkaloids, glycosides and saponins (Viswanatha *et al*., 2021). Flavonoids reduce blood pressure by decreasing endothelial cell oxidative stress, improving nitric oxide bioavailability, and modifying vascular ion channels (Maaliki *et al*., 2019). The ACE inhibitory activity has been associated to the flavonoids and terpenoids in medicinal plants (Guerrero *et al*., 2002). Further, polyphenols are widely distributed in natural foods and can reduce hypertension by decreasing vasoconstriction and regulating the renin-angiotensin-aldosterone system (RAAS) (Tain and Hsu, 2022). Thus it is proposed from current research, that the anti-hypertensive effect of *Colebrookea oppositifolia* through ACE inhibition can be attributed to the presence of the above-described phytochemical agents. However, there is still considerable room to explore the mechanism of action using various experimental models.

Conclusions and Recommendations

This research showed that a methanolic extract of *Colebrookea oppositifolia* leaves exhibited anti-hypertensive activity correlated to inhibition of the angiotensin-converting enzyme. *Colebrookea oppositifolia* extract can be advanced as a potential anti-hypertensive medicine. More studies are required to identify and separate the definite active component responsible for the anti-hypertensive activity.

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Novelty Statement

A methanolic extract of Colebrookea oppositifolia leaves, lowers blood pressure by inhibiting the angiotensin-converting enzyme (ACE).

Author's Contribution

Dr. Rukhsana Anwar: Study concept and design, results analysis, data interpretation, manuscript writing, correspondence and final approval.
Muhammad Usman Khalil: Performing of the experiments, results analysis, manuscript writing, and final approval.
Kanwal Ashiq: Analysis of the results, data interpretation, manuscript writing, final draft editing, and final approval.
All authors read and gave final approval of the draft.

Ethical statement
The Animal Ethics Board of the University College of Pharmacy, University of the Punjab, Lahore, Pakistan (Diary no. AEC/PUCP/1059) granted ethical approval.

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Conflict of interest
The authors do not have any conflicting interests.

References

Abdulazeez, M.A., and B.G. Kurfi. 2016. Isolation, partial purification and characterization of angiotensin converting enzyme from rat (*Rattus norvegicus*) lungs. Bayero. J. Pure. Appl. Sci., 9(2): 24-29. https://doi.org/10.4314/bajopas.v9i2.5

Abdulazeez, M.M., T.C. Ndubuisi, I. Mohammed, S.A. Abdullahi, W. Chintem and A.M. Wudil. 2013. Partial-purification and characterization of angiotensin converting enzyme inhibitory proteins from the leaves and seeds of *Moringa oleifera*. Int. J. Biochem. Res. Rev., 5(1): 39-48. https://doi.org/10.9734/IJBCRR/2015/12065

Al-Shabanah, O.A., O.A. Badary, M.N. Nagi, N.M. Al-Gharably, A.C. Al-Rikabi and A.M. Al-Bekairi. 1998. Thymoquinone protects against doxorubicin-induced cardiotoxicity without compromising its antitumor activity. J. Exp. Clin. Cancer Res., 17(2): 193-198.

Ashiq, S., and K. Ashiq. 2020. Genetic perspective of the congenital heart disease. Pak. Heart J., 53(3): 2020. https://doi.org/10.47144/phj.v53i3.1982

Ashiq, S., and K. Ashiq. 2020. In-silico analysis

of PON1, PON2 and PON3 genes role in coronary artery disease. Int. J. Comput. Biol. Drug Des., 13(4): 415-423. https://doi.org/10.1504/IJCBDD.2020.10033059

Ashiq, S., and K. Ashiq. 2021. The association of apolipoprotein-E (APOE) gene polymorphisms with coronary artery disease: A systematic review and meta-analysis. Egypt. J. Med. Hum. Genet., 22: Article number: 16. https://doi.org/10.1186/s43042-021-00135-2

Ashiq, S., K. Ashiq, S. Shabana, S.U. Shahid, M. Qayyum and H. Sadia. 2019. Prevalence and role of different risk factors with emphasis on genetics in development of pathophysiology of coronary artery disease (CAD). Pak. Heart J., 52(4): 279-287.

Beevers, G., G.Y. Lip and E. O'Brien. 2001. The pathophysiology of hypertension. Br. Med. J., 322(7291): 912-916. https://doi.org/10.1136/bmj.322.7291.912

Carson, P.E., 2000. Rationale for the use of combination angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker therapy in heart failure. Am. Heart J., 140(3): 361-366. https://doi.org/10.1067/mhj.2000.109215

Chatelain, C., V. Bänninger, C. Habashi, D. Wet, L. Gautier and R. Spichiger. 2006. Checklist of the flowering plants of sub-Saharan Africa. An index of accepted names synonyms.

Cienfuegos-Jovellanos, E., M.D.M. Quiñones, B. Muguerza, L. Moulay, M. Miguel and A. Aleixandre. 2009. Anti-hypertensive effect of a polyphenol-rich cocoa powder industrially processed to preserve the original flavonoids of the cocoa beans. J. Agric. Food Chem., 57(14): 6156-6162. https://doi.org/10.1021/jf804045b

Danish, P., Q. Ali, M.M. Hafeez and A. Malik. 2020. Antifungal and antibacterial activity of aloe vera plant extract. Biol. Clin. Sci. Res. J., 2020(1): e004-e004. https://doi.org/10.54112/bcsrj.v2020i1.4

Guerrero, M.F., P. Puebla, R. Carrón, M.L. Martín, L. Arteaga and L.S. Román. 2002. Assessment of the antihypertensive and vasodilator effects of ethanolic extracts of some Colombian medicinal plants. J. Ethnol., 80: 37–42. https://doi.org/10.1016/S0378-8741(01)00420-2

Hebert, L.A., M.E. Falkenhain, N.S. Nahman F.G. Cosio and T.M. O'Dorisio. 1999. Combination ACE inhibitor and angiotensin II receptor antagonist therapy in diabetic nephropathy. Am. J. Nephrol., 19: 1-6.

Hussain, S., G. Murtaza and R.A. Qureshi. 2016. Floristic studies of angiosperms of Rawalakot Azad Jammu and Kashmir Pakistan. J. Anim. Plant Sci., 26(6): 1696-1709.

Latif, A., K. Ashiq, M. Qayyum, S. Ashiq, E. Ali and I. Anwer. 2019. Phytochemical and pharmacological profile of the medicinal herb: Bryophyllum pinnatum. J. Anim. Plant Sci., 29(6): 1528-1534.

Maaliki, D., A.A. Shaito, G. Pintus, A. El-Yazbi and A.H. Eid. 2019. Flavonoids in hypertension: A brief review of the underlying mechanisms. *Curr. Opin. Pharmacol.*, 45: 57-65. https://doi.org/10.1016/j.coph.2019.04.014

Madhavan, V., D.K. Yadav, M. Gurudeva and S. Yoganarasimhan. 2011. Pharmacognostical studies on the leaves of *Colebrookea oppositifolia* Smith. Asian J. Tradit. Med., 4: 134-144.

Mushtaq, A., S. Rehmat, M. Jamil, A. Bashir, H. Anwar and S. Rashid. 2017. Evaluation of hepatoprotective and hepatotoxic activities of root extracts of cyperus pertenuis in rats. J. Pharm. Res. Int., 15(6): 1-12. https://doi.org/10.9734/BJPR/2017/32270

Owolabi, O.J., E.K. Omogbai and O. Obasuyi. 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia africana (Bignoniaceae) stem bark. Afr J. Biotechnol., 6(14): 1677-1680.

Pallab, K., B. Kush, P. Kumar, T. Girraj, T. Kishor, N. Singh and G. Shivani. 2011. *In vitro, in vivo* evaluation of cardioprotective effect of the leaf extract of *Colebrookea oppositifolia* Sm. J. Glob. Trend. Pharm. Sci., 2: 310-324.

Qasim, S., Alamgeer, M. Saleem, N.H. Alotaibi, S.N.A. Bukhari, K.S. Alharbi, H.M. Irfan and R. Anwar. 2021. Appraisal of the antiarthritic potential of prazosin via inhibition of proinflammatory cytokine TNF-α: A key player in rheumatoid arthritis. ACS Omega, 6(3): 2379-2388. https://doi.org/10.1021/acsomega.0c05698

Shlipak, M.G., W.S. Browner, H. Noguchi, B. Massie, C.D. Frances and M. McClellan. 2001. Comparison of the effects of angiotensin converting–enzyme inhibitors and beta blockers on survival in elderly patients with reduced left ventricular function after myocardial infarction. Am. J. Med., 110: 425-433.

Singh, R., 2015. Medicinal plants: A review. J. Plant

Sci., 3(1): 50-55.

Tabassum, N., and F. Ahmad. 2011. Role of natural herbs in the treatment of hypertension. Pharmacogn. Rev., 5(9): 30. https://doi.org/10.4103/0973-7847.79097

Tain, Y.L., and C.N. Hsu. 2022. Novel insights on dietary polyphenols for prevention in early-life origins of hypertension: A review focusing on preclinical animal models. Int. J. Mole. Sci., 23(12): 6620. https://doi.org/10.3390/ijms23126620

Venkateshappa, S.M., and K.P. Sreenath. 2013. Potential medicinal plants of Lamiaceae. Int. J. Res. For. Appl. Natl. Sci., 3(1): 82-87.

Viswanatha, G.L., H. Shylaja, H.Y. Kumar, M.V. Venkataranganna and N.B.L. Prasad. 2021. Traditional uses, phytochemistry, and ethnopharmacology of *Colebrookea oppositifolia* Smith: A mini-review. Adv. Trad. Med., 21(2): 209-229. https://doi.org/10.1007/s13596-020-00513-y

Yadav, D.K., 2019. Pharmacognostical, phytochemical and pharmacological profile of *Colebrookea oppositifolia* Smith. J. Drug Deliv. Ther., 9(6-s): 233-237. https://doi.org/10.22270/jddt.v9i6-s.3745