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ISOLATION, CHARACTERIZATION, AND ANTIHYPERTENSIVE ACTIVITY ALKALOIDS EXTRACTED FROM THE LEAVES OF THE ALSTONIA SCHOLARIS PLANT

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Abstract.

The study aims to investigate the Isolation, Characterization & Antihypertensive Life of Natural Alkaloids out of certain Selected Plants. The *Alstonia scholaris* papers used in this study are generally available in the tropics and can be obtained in Asia. The plant sample was verified by the pharmacognosy and pharmacology department. The powdered leaves of *Alstonia scholaris* (500 gm) are macerated using 1% HCl (pH 2) at space temperature overnight. After that, the combination was produced alkaline by putting 25% NH4OH solution (pH 9). The combination's color changed from the red wine to the black. The alkaline mixture was then bounced satisfactorily and purified using Whatman filter paper. Four fractions (15-19) were collected from column chromatography. All the fractions have shown the same Rf value in the TLC fingerprint, therefore they are incorporated established on TLC analysis generated in Hexane: Ethyl acetate (14:6). Nitric oxide synthase inhibitor, i.e. N-nitro-L-arginine methyl ester was used to produce hypertension in rats in (40 mg/ml/kg, i.p.). Every day, it is solubilized in 0.9 per cent NaCl solution. Colourless powder compound was obtained (yield 0.4%) and having MP 132-1340 C. Rf value in (Hexane: Ethyl acetate,65:35) at 0.55, UV–Vis λmax in methanol: (nm) 297, IR (KBr), m 913 (N–H bending), 1260 (C-N Stretching), 1396 (C–N), 1165, 1259 (-C-O- stretching) 1396, 1464 (C=C, Ar.), 2831, 2928 (C-H, Aliphatic) and 3564, 3315 (N-H Stretching). The 1H NMR spectrum also portrayed the distinctive peaks for various chemical compounds. The peak of 7.28-8.85 ppm was due to multiple aromatic protons. The 6.94-7.04 ppm peaks were characteristic of ethylene amino protons, and the 1.57-2 ppm peaks were allocated to alcohol protons. L-NAME significantly elevated MABP, SBP, and DBP in pentobarbital-anesthetized rats but not HR. The mean arterial blood pressure, systolic blood pressure and diastolic blood pressure of pentobarbital-anaesthetized L-NAME caused hypertensive rats do not alter after a single intragastric injection of the isolated alkaloid. Finally, isolated alkaloids from *Alstonia scholaris* supplement had antihypertensive properties in hypertensive rats.

Key words. Isolation, characterization, antihypertensive activity, natural alkaloids, selected plant, *Alstonia scholaris*.

Introduction.

Hypertension, often known as high blood pressure, is the most prevalent reason for going to the doctor, with over twenty million visits every year. Hypertension affects persons of all ages, from children to the elderly, with higher rates in the elderly, particularly those aged 50 and up. High blood pressure, often known as hypertension, is the most common cardiovascular illness, afflicting nearly one billion people worldwide and being a primary cause of morbidity and mortality [1,2]. Chronic diseases are expected to account for over three-quarters of all deaths by 2020, with 71 percent of deaths owing to cardiovascular disease and 75 percent of deaths due to stroke occurring in developing countries. Hypertension affected roughly 26.4 percent of the worldwide people in 2000, and it is rising at an alarming rate, with a predicted 60 percent by 2025. It is tough to diagnose high blood pressure because it does not have manifestations. Thus, many individuals' hypertension is not generally properly made due. A few cardiovascular issues, including [3,4], myocardial dead tissue, arteriosclerosis, end-stage renal infection, congestive cardiovascular breakdown, coronary illness, and stroke, are connected to hypertension. It additionally causes variant renal capacity, which prompts renal disappointment [5-7].

The present study's findings would be extremely beneficial to researchers because they attempt to fill a gap in the existing literature by providing new insights into the spectrum of all plants used for antihypertensive activity and active phytoconstituents, thereby bolstering ongoing research and development as a preventive and disease-modifying agent using novel delivery strategies.

Materials and Methods.

Identification and collection of plant material:

The Identification of *Alstonia scholaris*, commonly called blackboard tree, scholar's tree, milk tree or devil's tree in English [7], is a tropical evergreen tree in the Apocynaceae family. It is native to southern China, tropical Asia (especially the Indian subcontinent and Southeast Asia) and Australia, where it is a popular ornamental plant. It is a poisonous plant, but has been traditionally used for a variety of ailments and complaints. Called "Saptaparna" in India, it is the sacred tree of the Than Jain Tirthankar Ajitnatha. A vigorous tree prefers well-drained soil. Matures in 8–10 years. Propagated by cuttings (which root readily in sand), and seeds (collected from mature, undivided roots).

The *Alstonia scholaris* papers used in this study are generally available in the tropics and can be obtained in Asia. The plant

sample was verified by the pharmacognosy and pharmacology department. The voucher specimen was stored for potential use in the future. *Alstonia scholaris* were collected, washed, and dried at 40ºC for one hour, followed by drying at room temperature. After complete drying, the leaves were pulverized by a mechanical grinder. After that, the powdered material was sieved at 40 mesh and stored in an airtight container. The alkaloids were extracted from the dried powdered substance.

Isolation and characterization of alkaloid:

Overnight at ambient temperature, 500 grams of powdered *Alstonia scholaris* leaves were macerated in 1% HCl (pH 2). After that, 25% NH4OH solution (pH 9) was added to the mixture to turn it alkaline. The mixture turned from red wine to black color. After giving the alkaline combination a good shake, Whatman filter paper was used to filter it. Chloroform was used to extract the concentrated filtrate in stages. Ultimately, the chloroform extract was dried by evaporating it at 300 degrees Celsius, yielding 19.2 grams of dried residue. Column chromatography was used to separate the residue. The solution was being chromatographed above a silica gel column in a nutshell (60-80 mesh). On the column, enough time was allowed for fraction segregation and stabilization. After leaving the solvent in the column for 10 minutes to allow proper partitioning, the first elution was performed with hexane (50 ml). At a rate of 20-25 drops per minute, each 10 ml fraction was gathered in 5 test tubes. The collected fractions were exposed to chromatography for the isolation of alkaloids [8].

Isolation of active constituents:

Four fractions (15-19) were collected from column chromatography. The fractions were merged based on TLC analysis developed in Hexane: ethyl acetate (14:6) since all fractions displayed the same Rf value in the TLC fingerprint. The chemical tests (Mayer, Dragendroff, and Wagner) were run on TLC plates in a saturated iodine chamber to determine the constituent parts. The concentrated fraction was refrigerated for crystallization overnight, and the crystallized compound's m.p. was tracked [9].

Characterization of Isolated Alkaloids:

A UV/Visible in C2H5OH was used to quantify the UV spectrum at room temperature carefully. TLC was purposefully carried out using 0.25 mm-wide, thick Silica gel G plates (CDH, New Delhi). The TLC was detected by their UV fluorescence and by iodine vapour. Silica gel 60-120 mesh was used for column chromatography (CDH, New Delhi). Softening focuses are not really set in stone utilizing open vessels on a Cintex dissolving point mechanical assembly. The IR range was recorded on the range FTIR spectrometer. 1H NMR range was estimated on 400 MHz spectrometers involving TMS as an inside norm. The substance shifts were accounted for in ppm (δ). mass spectrometer filtered in a mass range [10].

Antihypertensive Activity if Isolated Constituents.

Experimental Rats:

Wistar rats strain of male gender (200-250 g) are being acquired from Animal Care. They were kept in conventional laboratory settings with free access to nourriture.

L-NAME Induced Hypertension:

L-NAME in a dose of 40 mg/ml/kg, i.p., was used to cause hypertension in lab rats in an experiment. Day after day, the L-NAME solution was prepared with 0.9 percent NaCl saline solution.

Experimental Procedure:

To anesthetize the rats, Sodium pentobarbital in a dose of 50 mg/kg, i.p. was used. To aid spontaneous respiration, the trachea was exposed and cannulated, as well as the left carotid artery. The stomach was intubated in order to administer the medicines intragastrically. A pressure transducer connected to a Power Lab system with a Chart program (AD Instruments) was used to record the blood pressure directly by carotid artery cannulation. Softening focuses are not really set in stone utilizing open vessels on a Cintex dissolving point mechanical assembly. The IR range was recorded on the FTIR spectrometer. 1H NMR range was estimated on 400 MHz spectrometers involving TMS as an inside norm. The substance shifts were accounted for in ppm (δ). mass spectrometer filtered a mass range [11-13].

Statistical analysis:

Statistical analysis was carried out using Sigma Stat and two-way repeated-measures ANOVA (version 3.5). P-values less than 0.05 were considered as a Statistical significance.

Results and Discussion.

Identification of alkaloid: Colorless powder compound with MP 132-134°C was obtained (yield 0.4 percent). The ultra-violet spectrum of alkaloid in methanol solvent shows a band at 297nm can be attributed to π-π transition.

The FT-IR spectrum (as KBr disc) shows all the expected bands in fingerprint and other regions. The asymmetrical and symmetrical aliphatic C-H are responsible for the strong bands in the FTIR spectra at 2928 and 2831 cm-1. Furthermore, it is possible to trace the stretching of the N-H bond to a broad band centred at 3564 cm-1 and its bending vibration to a medium band positioned at 913 cm-1. The symmetrical and asymmetrical aromatic C=C bonds can be used to explain the two strong bands at 1464 and 1396 cm-1, respectively. The stretching vibration bands of the C-N and C-O bonds result in two strong bands at 1260 and 1265 cm-1.

On the other hand, ^{1}H NMR spectrum of alkaloid compounds shows the following signals in a hexane: ethyl acetate mixture as a solvent: (2:1) (N-H Stretching). 8.58 (4 H, s, H-1, H-3, H-5, H-8), 7.2 (1 H, m, H-4), 7.07 (1 H, m, H-6), 6.94 (1 H, m, H-7), 3.39 (N-H), and 2.4 (H-O), 2- naphthyl amino ethanol [8-10].

Column fractionation and chromatographic profile of chloroform extract of leaves and their Rf values showed alkaloid isolation in the chloroform extract. The isolated compound showed a single spot in the chromatogram revealed the isolated compound is single phytoconstituents and has Rf value 0.55 in Hexane: Ethyl acetate (65:35). It has shown the same after being kept in a saturated iodine chamber. Isolated compound treated with alkaloids tests positive. It was crystallized, and the melting point was found 134°C. The UV spectrum λmax (CH3OH) revealed an absorption band at 297 nm. A colorless powder compound has sharp MP 132-134°C, and one spot in TLC (R_f = 0.55). The removed compound was portrayed and

affirmed dependent on unearthly investigations (^{1}H-NMR, IR, and Mass spectra).

The atomic equation of $C_{12}H_{13}ON$ (47.2) and 187 [M]+. The IR spectrum of compound 2-naphthyl amino ethanol has an absorption in the region 3565-3315 cm1 indicated the presence of NH. The ^{1}H NMR spectrum of the compound showed a signal for NH protons at δ four ppm, whereas the 2-naphthalene ethyl and alcohol groups were easily detected at their characteristic chemical shifts.

The IR spectra of the compound exhibited broad and medium intensity bands near 3565-3315 cm-1 due to NH and hydrogen-bonded OH, respectively. The intense bands at 2928-2831 cm-1 represented the asymmetric C-H stretching of the solvent. The corresponding bending vibrations appeared at 1363, 1396 and 1338, respectively. The presence of the aromatic system was confirmed by the combination of bands and aromatic overtones that appeared in the 2331, 1836, 1793, and 1741 areas. The 1678, 1645, 1546, 1539, and 1516 cm-1 bands aligned with the aromatic system's skeletal vibrations. Bending vibration in the O-H plane was responsible for the bands at 1260 and 1132 cm-1. The substitution pattern is corroborated by substantial C-H out-of-plane bending absorption at 913 cm-1 (Figures 1 and 2).

Also visible in the 1H-NMR spectra were the distinctive peaks associated with various chemical substances. Various aromatic protons caused the peaks between 7.28 and 8.85 ppm. The 1.57–2 ppm range peaks were attributed to alcohol protons, whereas the 6.94–7.04 ppm peaks were typical of ethylene amino protons. Owing to trans protons or the ethylene group, two doublets in the 7.5-8 ppm range with a coupling constant of 15–16 Hz were highly distinctive.

The specific protons in the compound were also represented by other peaks in the ^{1}H-NMR spectra of various compounds. The exact mass or fragmentation daughter peak is shown at 187 [M]+ confirms the compound of the desired alkaloid with molecular formula $C_{12}H_{13}ON$ [11-13].

The Effect of both Arterial BP and HR of Anesthetized Hypertensive lab Rats:

Alstonia scholaris elevated all different blood pressures in pentobarbital-anesthetized rats, but not HR (Figure 3). The mean arterial blood pressure, systolic blood pressure, and diastolic blood pressure of pentobarbital-anesthetized *Alstonia scholaris* caused hypertension in rats did not change after a single intragastric injection of the isolated alkaloid (4, 8, and 32 g/20 ml/kg). Compared to control, an alkaloid (16 g/20 ml/kg) effectively reduced high MABP and DBP 90 minutes after treatment (DDD water). When compared to control, an isolated

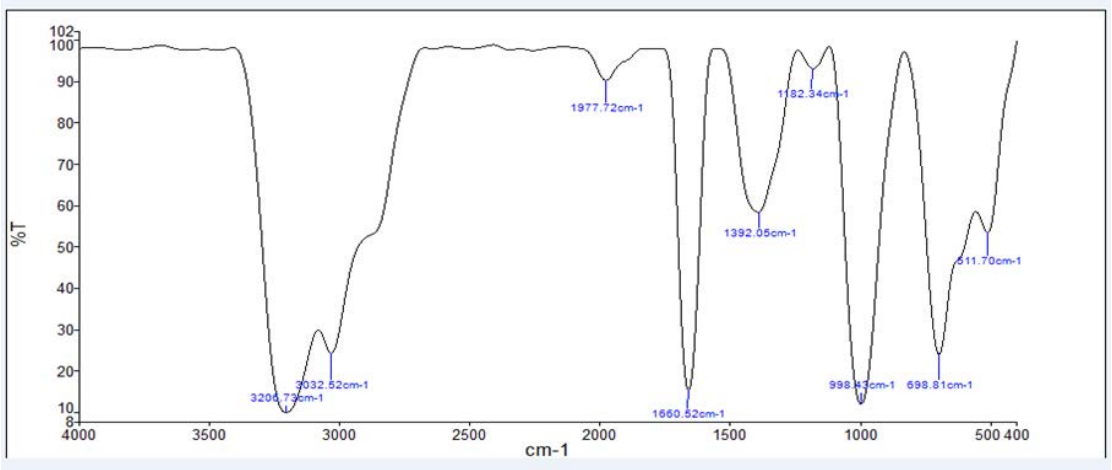

Figure 1. *FT-IR spectrum of isolated alkaloid.*

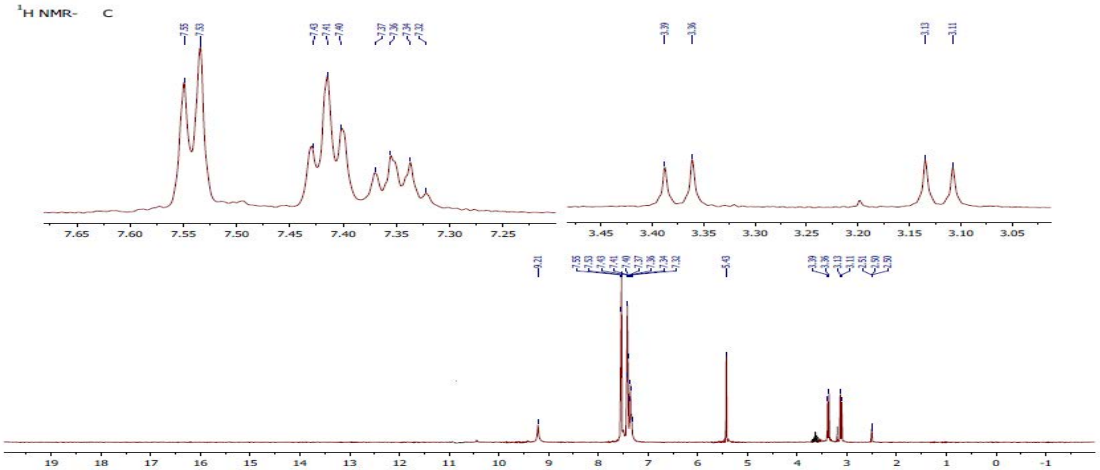

Figure 2. *The ^{1}H-NMR spectrum of alkaloid.*

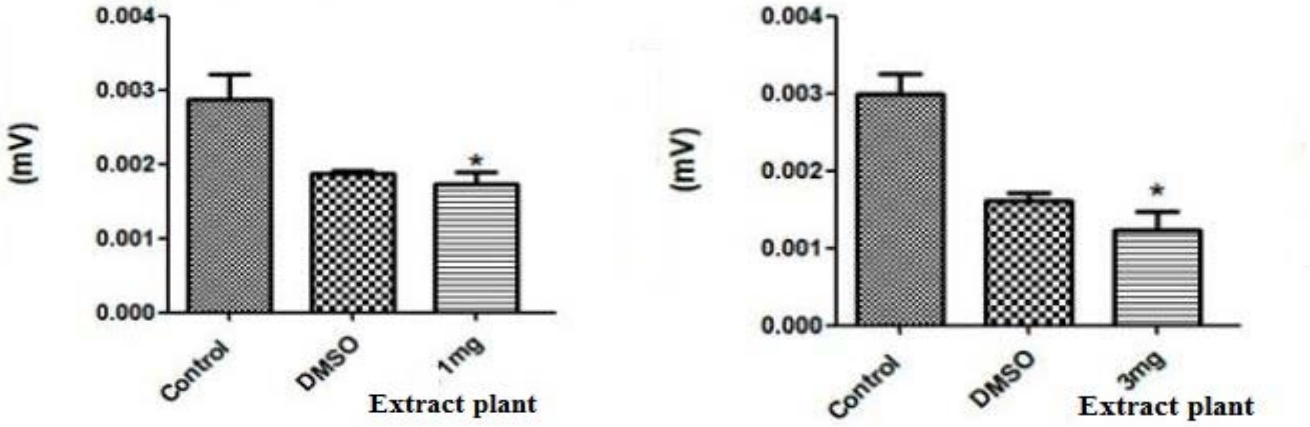

Figure 3. *The effects of individual alkaloids on MABP in hypertensive rats that had been anaesthetized.*

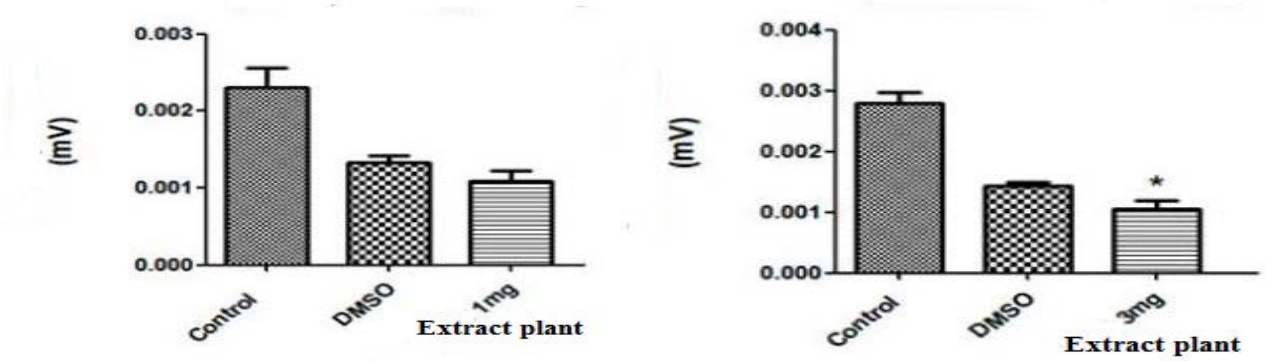

Figure 4. *SBP of anesthetized hypertensive lab rats after exposure to isolated alkaloids.*

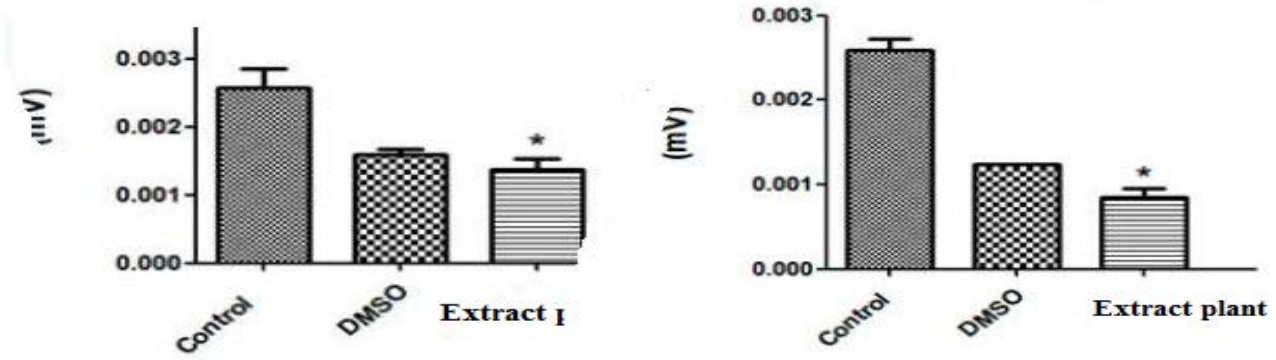

Figure 5. *Effects of individual alkaloids on systolic blood pressure in anesthetized hypertensive rats.*
Black square, black triangle and lead triangle represent the isolated alkaloid at 16 and 32 g/kg orally and the standard flavonoid i.e. quercetin at 5 mg/kg.
In our study, we found that the isolated alkaloid at 16 and 32 g/kg orally significantly and moderately reduced blood pressure compared to the disease control group. Similarly, quercetin at 5 mg/kg also significantly reduced blood pressure compared to the disease control group.

alkaloid (16 g/20 ml/kg) significantly reduced high SBP throughout a 75–90-minute timeframe (DDD water). Compared to control, the high MABP decreased significantly from 10 to 90 minutes after Quercetin (5 mg/20 ml/kg) was administered. Quercetin effectively reduced high SBP and DBP over the 45–90-minute interval following dosing. HR was unaffected by all dosages of different alkaloids and Quercetin [14-16].

In *Alstonia scholaris* incited hypertensive rodents, secluded alkaloid at centralization of 16 g/20 ml/kg (i.g.) and its flavonoid Quercetin at a 5 mg/20 ml/kg (i.g.) had antihypertensive impacts. The treatment of alkaloids brought about critical decreases in expanded MBP, SBP, and DBP in *Alstonia scholaris* actuated hypertensive rodents. These outcomes recommend that *Alstonia scholaris* affected L-NAME-incited hypertensive rodents [17,18].

Muangnongwa (2004) revealed that alkaloids (32 g/kg, p.o.) could bring down systolic blood pressure in deoxycorticosterone acetic acid derivation salt-actuated hypertension rodents yet had no effect in normotensive rodents.

In *Alstonia scholaris* incited hypertension rodents, the flavonoid quercetin had hypotensive properties. In *Alstonia scholaris* actuated hypertension rodents, quercetin significantly

decreased expanded MBP, SBP, and DBP. These discoveries add to late research that showed quercetin could assist hypertensive creatures with decreasing their circulatory strain. In *Alstonia scholaris* initiated hypertension rodents, segregated alkaloids and Quercetin treatment didn't influence pulse (Figures 4 and 5) [19-22].

Mechanism behind the antihypertensive:

The Elucidate the mechanism behind the antihypertensive effect of alkaloids extracted from Alstonia is *Alstonia scholaris* is an evergreen tree that is rich in indole alkaloids and has been used to treat lung diseases and several diseases. The primary indole compounds found in this plant are scholaricine, 19-epischolaricine, vallesamine, and picrinine, which have been shown to exert toxic effects on non-rodents [22].

Conclusion.

Finally, isolated alkaloids from *Alstonia scholaris* supplement were antihypertensive properties in hypertensive rats. *Alstonia scholaris* extract's antihypertensive action may be due in part to quercetin.

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Author's contributions.

This research was carried out with the help of a group of professors from the College of Pharmacy, Almaaqal University/ Iraq, and the authors from the College of Pharmacy, University of Basrah, Iraq.

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