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Anti-Hypertension Activity of *Leucas aspera* Extract by Molecular Docking Method

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Abstract:

- ***Background:***

Hypertension (high blood pressure) is a major cause of cardiovascular disease and is highly prevalent worldwide, particularly in developing countries.

- ***Aim & Objectives:***

Present investigation has been conducted to assess the anti-hypertensive property of Leucas aspera ethanolic extract through phytochemical screening, GC–MS analysis and molecular docking approach. In the initial part, a green ethanolic extract was prepared using the Soxhlet method, which comprised terpenoids, flavonoids, phenols, tannins, saponins and alkaloids. The GC–MS analysis revealed 2-Methoxy-4-vinylphenol as an important phytocompound that possessed high binding affinity (–6.4 kcal/mol) to the AT1 receptor through docking studies, making numerous hydrogen and hydrophobic bonds with vital residues. Based on these findings, it may be concluded that the plant Leucas aspera may exert antihypertensive effects by acting as an antagonist against the AT1 receptor.

- ***Material & Methods:***

Aerial plant (*Leucas aspera*) parts were washed, dried at 40°C, and ground into powder. This material underwent Soxhlet extraction using 70% ethanol at a 3:1 ratio for 6–8 hours. The process continued until the solvent became colourless, ensuring thorough extraction of soluble constituents. Finally, the solution was filtered, the solvent evaporated, and the resulting botanical extract was stored at 4°C.

- ***Result & Discussion:***

GC-MS analysis of the plant extract revealed various phytochemicals including 2-Methoxy-4-vinylphenol suggesting possible antihypertensive synergy. Molecular docking showed that 2-Methoxy-4-vinylphenol has a good affinity to Angiotensin II Type 1 (AT1) receptor (4YAY) with an affinity of \(-6.4 \text{ kcal/mol}\) and significant hydrogen bonds (ARG167) and hydrophobic interactions (TRP84, TYR35, VAL108). These data are consistent with an ARB-like antagonistic mechanism. Integrated in silico studies suggest this compound as a possible anti-hypertensive candidate that warrants further in vitro and in vivo validation.

- ***Conclusion:***

The identification of bioactive compounds from the plant through GC-MS analysis led to the discovery of 2-methoxy-4-vinylphenol. Docking studies conducted on the plant compound showed its ability to interact with residues of the Angiotensin II type 1 receptor (\(-6.4 \text{ kcal/mol}\)), including ARG167, TRP84, TYR35, etc., which shows that it is an antagonist to the receptor, and hence may be antihypertensive.

Keywords: *Leucas aspera, GC-MS Analysis and Molecular Docking Assay, Angiotensin II, AT1 Receptor.*

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I. INTRODUCTION

➢ *Hypertension:*

Blood pressure is the pressure of blood flow in the arteries, which are the main blood vessels through which blood leaves the heart. High blood pressure is where the blood pressure remains consistently high above what is considered healthy [1]. In this case, one is said to be suffering from high blood pressure or hypertension. Hypertension is a condition where the blood pressure is persistently high. In this case, there will be pressure put on the heart, brain and other body organs. The diagnosis for hypertension is when blood pressure is 140/90mmHg [2]. Blood pressure consists of two measurements: systolic and diastolic pressures. Systolic pressure is the pressure in the arteries during heartbeats. On the other hand, diastolic pressure is the pressure in the arteries between heartbeats. A normal blood pressure should be 120/80 mmHg [3].

- *Types:* Primary hypertension, Secondary hypertension.

➢ *GC-MS and Molecular Docking Assay:*

GC-MS is an analytical procedure employed to separate and analyze volatile components in complex samples including extracts from plants.[4] It helps obtain a comprehensive chemical profile through the combination of capabilities of gas chromatography and mass spectrometry.[5] Molecular docking is a computational approach that allows one to evaluate the interaction potential of small molecules with specific proteins/enzymes.[6] This can be considered as a virtual screening of drugs.[7]

In the proposed research project, GC-MS is applied to identify the phytochemical composition of the sample, while molecular docking helped to assess how these phytochemicals can interact with important proteins. Computer modeling and analytical techniques are chosen at the first stage since they allow for obtaining results rather quickly, affordably, and without involving animals or cells.[8]

II. PLANT PROFILE

➢ *Plant Description:*

Leucas aspera is an annual herb characterized by its strong, hairy, sharply four-angled stem that reaches 15 to 60 cm in height. The leaves are narrowly linear, slightly pubescent, and measure 8 cm long and 1.25 cm wide with entire or faintly serrate leaf margins. The petioles are short, having a length of 2.5 to 6 mm [9]. Leucas aspera bears small, white, sessile flowers in dense whorls at the apex or on the sides of branches. Each flower is subtended by thin bracts measuring 6 mm long; bristles are present at the tips of the bracts together with fringes of fine hairs. The tubular calyx measures 8 to 13 mm [10]; the upper half of the calyx is ribbed and pubescent. Narrow mouth and small triangular teeth characterize the calyx with the upper tooth larger than the others. The corolla is about 1 cm long, 5 mm pubescent tube, and ringed throat [11]. Upper lip is short and woolly while the lower lip is longer with short lateral lobes and broad median lobe. The fruit is composed of smooth brown oblong nutlets measuring 2.5 mm long, rounded outer face and angular inner face [12]

Fig 1 *Leucas aspera* - Aerial Parts

➢ *Botanical Classification:*

- Kingdom: Plantae (Plant).
- Subkingdom: Tracheobionta (Vascular plants).
- Superdivision: Spermatophyta (Seed plants).
- Division: Angiospermae.
- Class: Dicotyledonae.
- Subclass: Gamopetalae.
- Series: Bicarpellatae.
- Family: Labiatae or Lamiaceae.
- Genus: Leucas.
- Species: aspera.

III. METHODOLOGY

➢ *Collection and Authentication:*

The entire *Leucas aspera* plant was collected from the local area and authenticated by Dr. P. Radha, Research Officer (Botany), Siddha Medicinal Plant Garden, Mettur Dam, Tamil Nadu-636401.

The plants were washed three times with tap water and sterilized by spraying with 70% alcohol. The cleaned plant materials were dried in shade at room temperature to prevent chemical alterations.

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Once completely dried, the materials were ground into fine powder using a mortar and pestle or an electric grinder. The resulting coarse powder was extracted using a Soxhlet apparatus.

➢ *Extraction by Soxhlet Apparatus:*

The extraction of crude drugs from plants is one of the most studied areas in pharmaceutical research. The selection of an appropriate extraction technique is based on the moisture content of the plant matter as well as the physical nature of the compound. In most cases, crude drug extracts are usually obtained using Soxhlet extraction with aqueous solvents [13].

There are three major components of this apparatus namely; the round-bottom flask for the solvent, the Soxhlet flask for the compounds being extracted, and the condenser for vapor condensation. We filled our Soxhlet flask with 100g of the aerial parts of the plant material. We put the solvent in the round bottom flask and carried out the extraction at reduced pressure. We maintained the temperature of 60-80°C to ensure that the solvent boiled under control of the heating mantle. Vapor moved through the drive tube into the condenser where it was condensed due to continuous flow of water in the condenser. [14]

The condensed solvent falls back on the packed material in the flask itself. The collection and extraction of material occur simultaneously in the flask. The colour of the solvent changed as the compound in the material dissolved in it. Thus, the crude plant material extract has been obtained, and it takes about 7-8 hours to complete the extraction. The solvent was evaporated, and finally, it yields a green extract, which was stored in the refrigerator for further use [15].

➢ *GC-MS (Gas Chromatography-Mass Spectrometry) Analysis:*

Gas chromatography-mass spectrometry (GC-MS) analysis was performed on a Shimadzu GC-QP2010SE device with an AOC-20i autosampler. Prior to each injection, the syringe was flushed with a pre-solvent, solvent, and sample three times, respectively. Plunger speed for suction and injection was set to high with a viscosity compensation time of 0.2 sec. Insertion and washing speed were both kept at high with a washing volume of 6 µL. The injection mode was normal with five pumping cycles and an injection port dwell time of 0.3 sec [16].

The GC parameters were as follows: the initial column oven temperature was 50 °C followed by heating to 280 °C with a rate of 6 °C/min. This temperature was then held at 280 °C for 2 min. The injector temperature was maintained at 250 °C with splitting ratio of 10:1. Helium was used as the carrier gas with linear velocity controlled at 39.7 cm/s. The column flow was 1.20 mL/min, the total flow was 16.2 mL/min, and the purge flow was 3.0 mL/min. The injection pressure was 68.1 kPa, and equilibration time was 0.5 min [17].

Mass spectrometer operated in EI mode with ion source temperature of 250 °C. The interface temperature was set to 200 °C. A solvent cut time of 3.5 minutes was used.[18]. The detector gain was set to 1000, with the gain mode at 0.00 kV. Data collection happened in full scan mode across the m/z range of 50 to 500, with a scan speed of 1666 scans per second and an event time of 0.30 seconds. The total run time was 35 minutes. [19].

➢ *Molecular Docking Assay:*

- *Software Used:*

✓ AutoDock 4.2.6 (MGL tools).
✓ AutoDock vina.
✓ BIOVIA Discovery Studio.

➢ *Preparation of Ligand:*

Phytochemical 2-Methoxy-4-vinylphenol (CID: 332 on PubChem) was acquired from PubChem database in 3D SDF format. Structure of 2-methoxy-4-vinylphenol was visualized using BIOVIA Discovery Studio and was then converted into PDB format. Ligand preparation involved AutoDock 4.2.6 software. In the course of ligand preparation, nine non-polar hydrogens were merged while three rotatable bonds were determined. Next, the PDB file of 2-Methoxy-4-vinylphenol was converted to PDBQT format by means of AutoDock 4.2.6. [20]

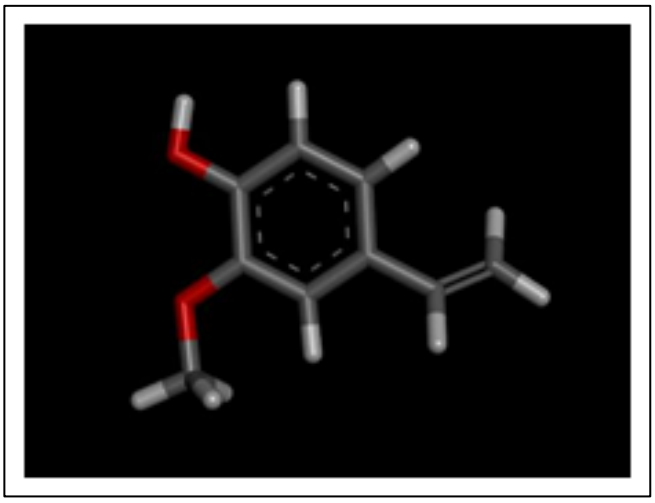

Fig 2 3D Structure of Phytocompound Named 2-Methoxy-4-Vinylphenol.

➢ *Preparation of Target Protein:*

The target protein chosen is the AT1 Receptor (PDB ID: 4YAY) which was crystallized using the X-ray diffraction method with a resolution of 2.90 Å and contains only one chain referred to as chain A. The PDB format for the target protein was sourced from the RCSB Protein Data Bank and viewed and optimized through BIOVIA Discovery Studio software. Target protein optimization included the elimination of heteroatoms and water molecules. Polar hydrogens were added and the active site was found out; the characteristics of xyz were recorded as 4YAY (-10.267726 10.245961 41.829908). Target protein was transformed from PDB to PDBQT using AutoDock 4.2.6 software. Molecular docking was carried out on the optimized target protein. Figure 2 shows the 3D structure of the target protein.

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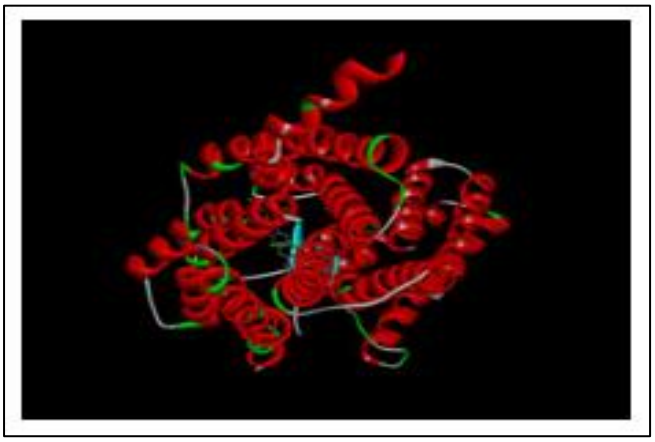

Fig 3 Structure of Target Protein.

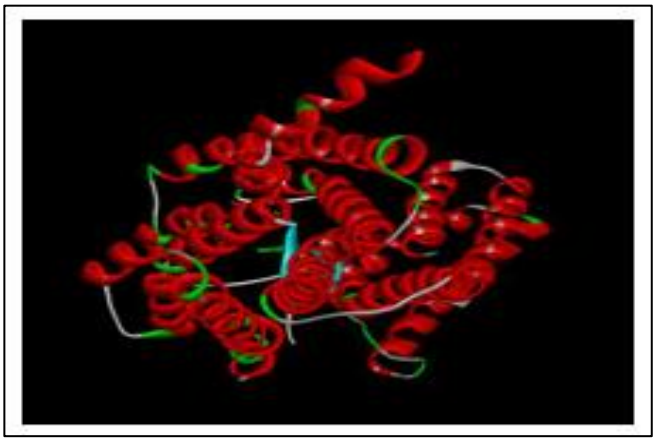

Fig 4 Structure of Optimized Protein

➢ *Molecular Docking Study:*

2-Methoxy-4-vinylphenol ligand, which had been optimized, was docked with the AT1 Receptor domain using AutoDock Vina. A configuration file was created for the purpose of docking the molecules. Figure 3 shows the optimized target protein.

Lamarckian genetic algorithm was used, where energy evaluation was done up to 2.5 million for molecular docking studies. The docking study was carried out by AutoDock Vina through command prompt. The best confirmation for docked complex was done through BIOVIA Discovery Studio. [21]

IV. RESULT

➢ *Phytochemical Screening:*

Table 1 Phytochemical Inference Data

Phytoconstituents	Inference
Flavonoids	+
Saponins	+
Phenol and Tannins	+
Carbohydrate	-

➢ *GC-MS (Gas Chromatography-Mass Spectroscopy): (Chromatogram)*

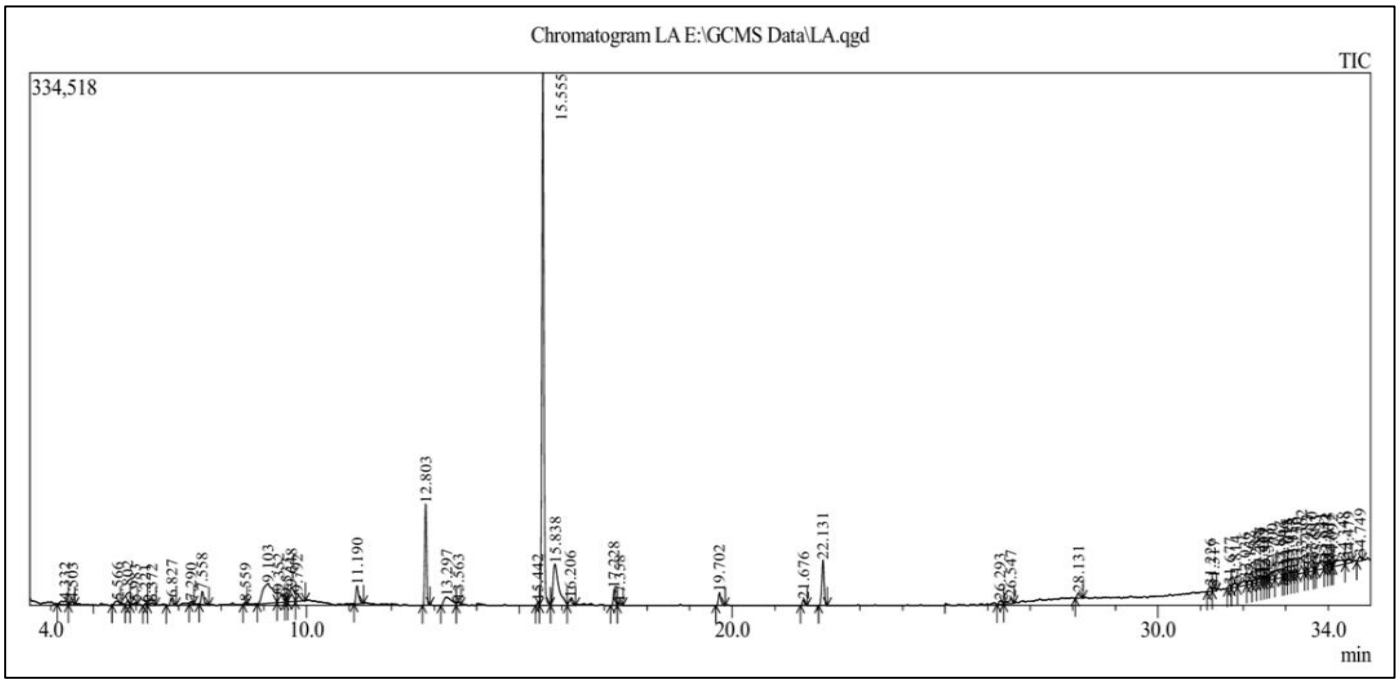

Fig 5 GC-MS (Gas Chromatography-Mass Spectroscopy): (Chromatogram)

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This chromatogram graph acts as a chemical fingerprint of the plant *Leucas aspera,* which highlights the presence of 2-Methoxy-4-vinylphenol

➢ *Molecular Docking:*

- *AT1 Receptor with 2-Methoxy-4-vinylphenol-*

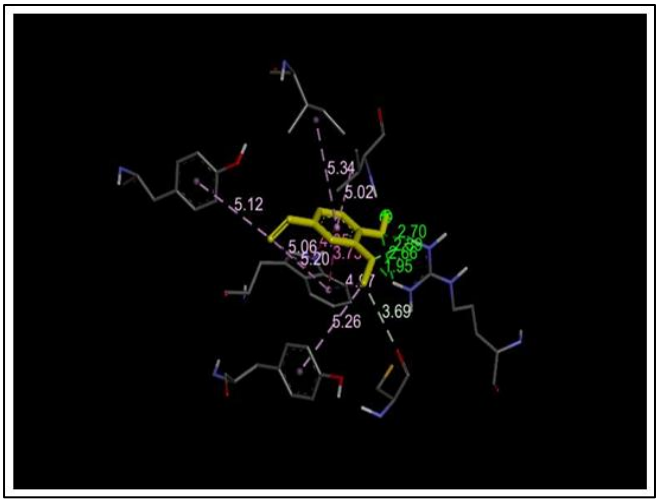

Fig 6 3D Image

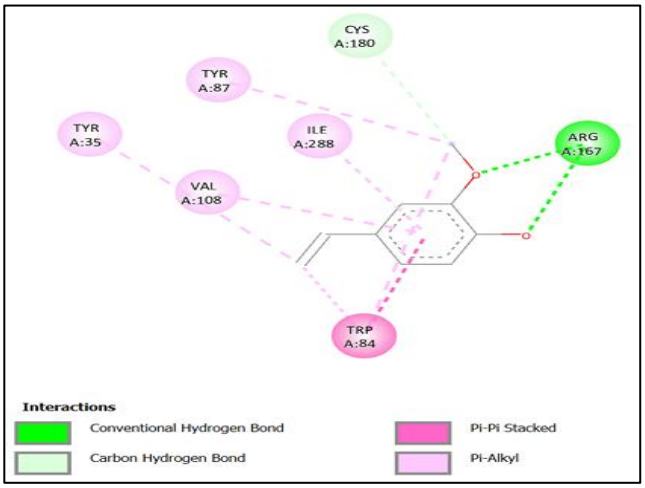

Fig 7 2D Image

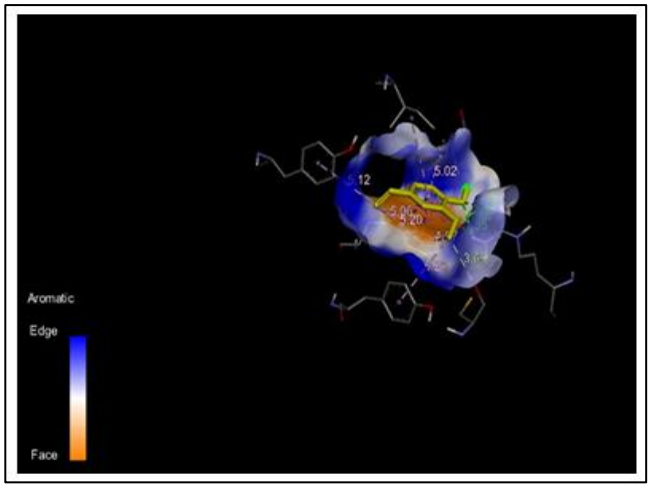

Fig 8 Aromatic

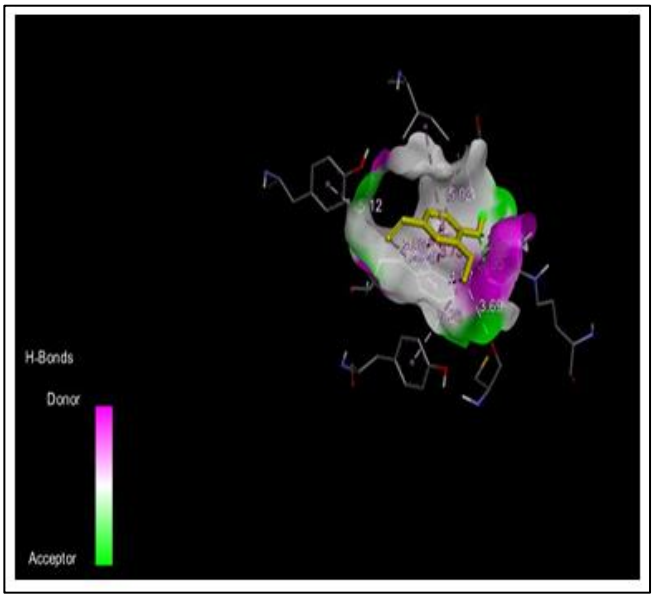

Fig 9 Hydrogen Bond

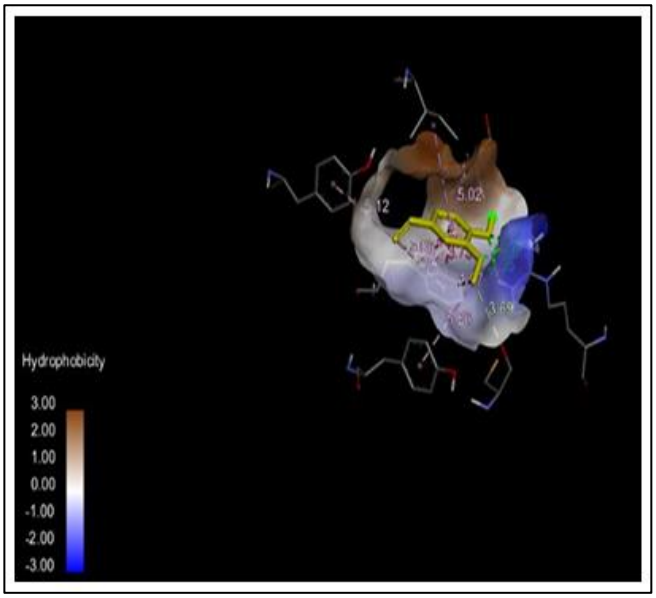

Fig 10 Hydrophobicity

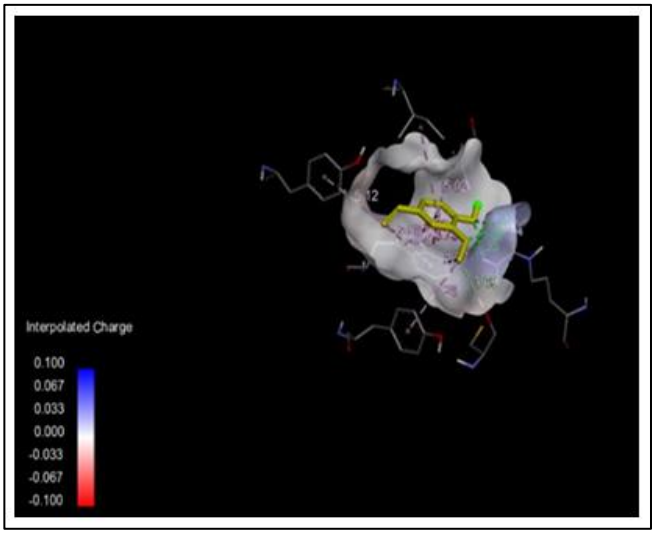

Fig 11 Charge

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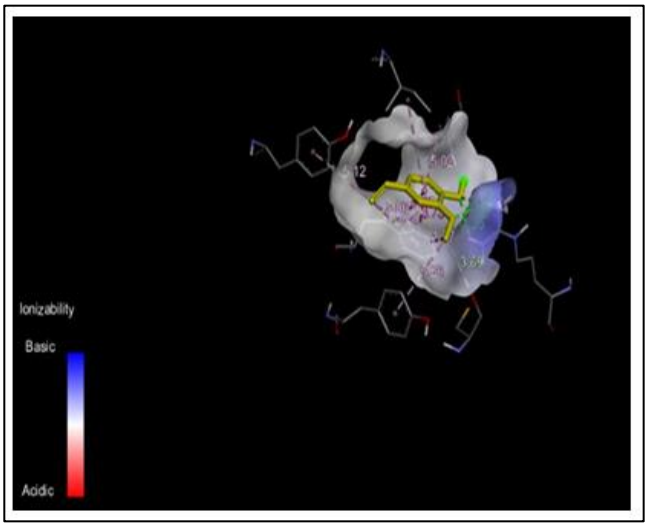

Fig 12 Ionizability

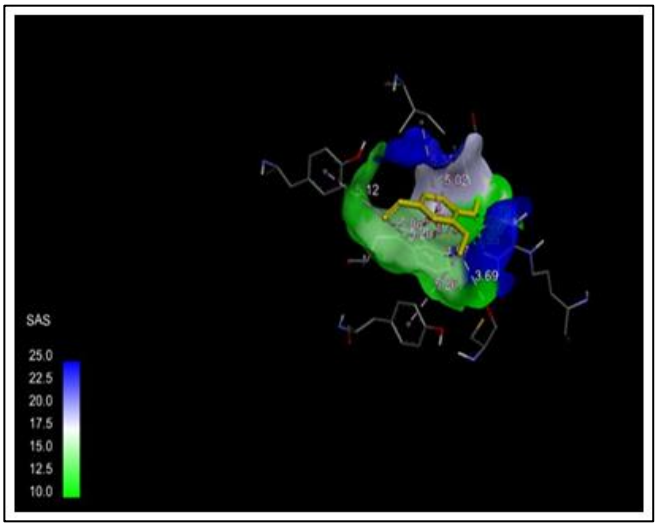

Fig 13 SAS

Table 2 AT1 receptor with 2-Methoxy-4-Vinylphenol Binding Affinity

S. No	Binding affinity	Bonds	Amino acid residues
4K8M- 2-Methoxy-4-vinylphenol-	-6.4 Kcal/mole	4 Hydrogen Bonds	A:ARG167:HH12 - :UNK0:O1- 1.95 Å A:ARG167:HH12 - :UNK0:O2 – 2.66 Å A:ARG167:HH22 - :UNK0:O1- 2.38 Å A:ARG167:HH22 - :UNK0:O2 – 2.69Å
		1 Carbon Hydrogen Bond	:UNK0:C10 - A:CYS180:O - 3.68 Å
		3 Hydrophobic Pi-Pi Stacked Bond	A:TRP84 - :UNK0 – 4.35 Å A:TRP84 - :UNK0 – 3.73 Å
		7 Hydrophobic Pi-Alkyl Bond	A:TYR35 - :UNK0:C11 – 5.11 Å A:TRP84 - :UNK0:C11 – 5.06 Å A:TRP84 - :UNK0:C11 – 5.19 Å A:TRP84 - :UNK0:C10 – 4.97 Å A:TYR87 - :UNK0:C10 – 5.26 Å :UNK0 - A:VAL108 – 5.02 Å :UNK0 - A:ILE288 – 5.34 Å

V. DISCUSSION

The extract comprises of flavonoids, saponins and tannins, providing an adequate phytochemical background to the plant extract, thus making it highly relevant in cardiovascular disorders.

The GC-MS analysis of the plant extract showed various phytochemicals such as 2-Methoxy-4-vinylphenol, diethyl phthalate, isoamyl nitrite and kauran derivatives. Out of all these, the 2-Methoxy-4-vinylphenol was selected for the MD studies due to its bioactivity and peak appearance in the chromatograph. Multiple bioactive phytochemicals indicate complex chemical nature of the extract and hence potential synergistic pharmacological effects.

MD of 2-Methoxy-4-vinylphenol with the AT1 receptor (PDB ID: 4YAY) gave a binding affinity of –6.4 kcal/mol. Four hydrogen bonds were formed between the ligand and ARG167 residue whereas one carbon-hydrogen bond was observed between ligand and CYS180 residue. Additionally, hydrophobic interactions were also seen with TRP84, TYR35, TYR87, VAL108 and ILE288 residues. These observations show that a stable conformer of the compound could be achieved inside the receptor cavity. Formation of hydrogen and hydrophobic bonds shows that the compound can stably bind inside the receptor pocket and prevent the binding of Angiotensin II.

The docking interactions observed have proven consistent with the pharmacology of Angiotensin II Type 1 (AT1) Receptor Antagonists, otherwise referred to as Angiotensin Receptor Blockers (ARBs). The ARBs inhibit vasoconstriction and reduce blood pressure by inhibiting Angiotensin II. Therefore, the in-silico results indicate that 2-Methoxy-4-vinylphenol is capable of acting as an antihypertensive. Although the presence of 2-Methoxy-4-vinylphenol was confirmed using GC-MS, the mode of action as a receptor antagonist was determined by Molecular Docking.

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In conclusion, the application of Gas Chromatography–Mass Spectrometry and Molecular Docking tests has been found to provide a simple and inexpensive method for correlating the phytochemical composition with their biological activities. Further validation studies will need to be carried out to prove the antihypertensive properties.

VI. CONCLUSION

From the GC-MS analysis of the plant extract, the following were the phytochemicals present; 2-Methoxy-4-vinylphenol, diethyl phthalate, isoamyl nitrite, and kauran derivatives. From the list of these phytochemicals, 2-Methoxy-4-vinylphenol was chosen for docking studies with Angiotensin II type 1 receptor due to its higher percentage and bioactivity. During the docking analysis, the binding energy was found to be -6.4kcal/mol due to hydrogen bonding and hydrophobic interactions with important amino acids residues including; ARG167, TRP84, TYR35, TYR87, VAL108, and ILE288. The result indicates that the phytochemical can be used to stabilize the binding site of the receptor and inhibits angiotensin II. Therefore, sufficient data has been generated to prove the relationship between phytochemistry and antihypertensive property.

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