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Research Article

Borassus Flabellifer Sap As ACE Inhibitors

# Identification of *Borassus Flabellifer* Sap Compounds Against Angiotensin-Converting Enzyme for Potential Antihypertensive Inhibitors

**M.Shobana[1]* and S.Thilagavathi[2]**

[1]*Department of Food Science and Nutrition, Periyar University, Salem, Tamil Nadu, India*

[2]*Assistant Professor, Department of Food Science and Nutrition, Periyar University, Salem, Tamil Nadu, India*

**Abstract:** On a global scale, cardiovascular problems have become more important recently. The existing treatments for high blood pressure are generally unsuccessful and painful for patients. Since it poses a significant risk to the cardiovascular system and the general public's health, hypertension should be carefully managed. Hypertension with a systolic reading is extremely harmful. As a result, there is increased interest in creating antihypertensive drugs with natural ingredients. The study's key finding is that *Borassus flabellifer* sap comprises significant bioactive components, including 9,10-Anthracenedione. The angiotensin-converting enzyme (ACE), which converts angiotensin I into the active peptide vasoconstrictor hormone angiotensin II, may be inhibited by *Borassus flabellifer*, according to an in-silico investigation. The 9,10-Anthracenedione and ACE molecular docking investigation revealed extremely low binding energy (-8.6 kcal/mol). One must be aware of the ACE inhibitors that are already on the market to choose an efficient ACE inhibitor. Docking interactions of 9,10-Anthracenedione hence strongly imply its potential as an antihypertensive substance. It is a possible bioactive *Borassus flabellifer* sap ingredient for the creation of nutraceuticals and functional foods, as well as a candidate for the development of an efficient antihypertensive medicine. According to the study's findings, *Borassus flabellifer* sap is a potent nutraceutical compound that can be used to treat antihypertensive.

**Keywords:** Angiotensin-Converting Enzyme, ACE Inhibitors, *Borassus Flabellifer,* Molecular Docking

***Corresponding Author**

**M.Shobana , 1Department of Food Science and Nutrition, Periyar University, Salem, Tamil Nadu, India**

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## 1. INTRODUCTION

Cardiovascular diseases have recently escalated in importance as a major global issue. The World Health Organization reports a rise in the prevalence of this illness. Aortic aneurysm, coronary heart disease, blood vessel abnormalities, stroke, and other cardiovascular illnesses are all worsened by hypertension, a significant risk factor [1]. Another cardiovascular issue is cardiac arrhythmia. Any irregular heartbeat resulting in a stroke is known as an arrhythmia. Heart rhythm abnormalities can be inherited or result from myocardial injury, which can occasionally be brought on by hypertension [2]. The current therapies for high blood pressure are often painful for people and are not particularly successful. This depends on the patient's meticulous control over the dosage and drug delivery time 3. Additionally, some individuals experience an adverse reaction following the treatment, which causes their blood pressure to drop quickly. Captopril is one of the most often prescribed drugs to treat hypertension. Like many others on the market, this medication was created using computational methods. Computational research and rational drug design have been crucial components in creating novel medications during the past few decades. Hypertension should be carefully addressed since it poses a severe risk to the cardiovascular system and public health. Systolic blood pressure is particularly dangerous. The renin-angiotensin system (RAS), one of the key systems responsible for increasing stress, can be inhibited to reduce blood pressure and enhance cardiovascular health [4]. Several extremely specialized enzyme processes control the RAS. Renin, produced in the kidneys and breaks down angiotensinogen to produce angiotensin I, initiates the first enzymatic step in the route. Angiotensin-converting enzyme (ACE) then breaks down angiotensin I to have the active peptide vasoconstrictor hormone angiotensin II [5].

*Fig.1. Borassus flabellifer L*

It is well known that several angiotensin-converting enzyme (ACE) inhibitors are effective in managing hypertension [6]. The exploration of compounds containing phosphorus is prompted by the hunt for ACE inhibitors devoid of the sulfhydryl group. Similar to enalapril, phosphinic acid can bind to ACE. Similar to how sulfhydryl groups interact with phosphinic acid, zinc atoms do the same. Through docking experiments, the capacity of various derivatives of *Borassus flabellifer* that operate as ACE inhibitors is determined. The derivative with the highest potency may lead the way to develop novel ACE inhibitors with the potential to suppress the activity.

## 2. MATERIALS AND METHODS

### *2.1 Sap Collection, preservation*

The palm plant sap is a clear, pleasant material obtained by tapping the flowers of *Borassus flabellifer*. Recently, its potential therapeutic uses have come to light due to several significant phytochemical components. In order to prevent fermentation or spoilage, Borassus flabellifer sap was collected twice daily (at night and during the day) from the Elimedu area of Thiruchengode, Namakkal district. It was maintained in the freezer at a temperature of -80°C. Dr.M.U.Sharief, Scientist 'E' & Head of Office, Botanical Survey of India, Southern Reginal center, Coimbatore-641003 has authenticated sap of *Borassus flabellifer*. The voucher number BSI/SRC/5/23/2021/Tech/182 dated: 25.10.2021.

### *2.2 Lyophilization of Sap*

After, collected *Borassus flabellifer* samples were freeze-dried and conserved at 4◦ C for GC/MS analysis[7]. Lyophilization is a dehydration technique primarily used to stabilize the therapeutic agents from the aqueous solutions. Eliminating the water molecule from the product significantly extends the shelf-life. Most of the approved bio-products are stabilized by the Lyophilization method [8]. After loading the vials, the shelf temperature was reduced gradually to freeze the liquids. After that chamber pressure (Pc) was decreased (70 to 1 Pa) to establish the primary drying phase, enabling the sublimation of all the ice and the formation of a porous network[9-10].The optimum condition of the freeze-drying process was -20°C for 3 hours to pre-freeze, -20°C for 4 hours for first drying, -10°C for 7 hours is for second drying, and finally 0°C for 6 hours for third drying, then it was heated up to 30°C. The sap powder preparation method followed in this study is already patented by Shobana. M and

Thilagavathi.S in the year 2020, this method helps to convert the 12g nutrient powder from the 100ml of natural plant extracts [11] (*Borassus flabellifer*).

### *2.3 Phytochemical analysis*

#### 2.3.1 Test for flavonoids

To 1 ml of plant extract mixed with a few drops of dilute sodium hydroxide development of an intense yellow color solution, which in turn became colorless upon addition of a few drops of dilute acid indicates the presence of flavonoid in plant extract [11].

### *2.4 Test for Saponin*

Briefly, 50mg of plant extract was mixed with 20ml of distilled water, and it vortexed in a graduated tube for 15 minutes. The formation of 1cm foam layer indicates the presence of saponin [12.]

### *2.5 Test for Phenol*

A few drops of 1% lead acetate added to the 2ml of plant extract resulted in a bulky white precipitate, indicating the presence of phenolic compounds in the plant extract sample [13].

### *2.6 Test for Glycosides*

A few drops of ferric chloride and concentrated sulphuric acid added to the plant extract in glacial acetic acid turn the reaction mixture into reddish brown at the junction of two liquids and bluish green color in the upper layer of liquid [14].

### *2.7 Test for Tannin*

To 1ml of plant extract, add 1 ml of distilled water and a few drops of ferric chloride solution added in the reaction mixture. The blue and green color formation indicates the presence of gallic and catecholic tannin, respectively in the plant extract [15].

### *2.8 Test for alkaloid*

The plant extract solution was dissolved in dilute hydrochloric acid and filtered using Whatman No 1 filter paper. Then the filtrate was treated with Mayer's reagent (potassium mercuric iodide). The formation of yellow color precipitate in the reaction mixture confirms the presence of alkaloids .

### *2.9 GCMS analysis*

Under the following conditions, the extract was analyzed using a GC-MS Clarus 500 Perkin Elmer system with an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer[16]: With an injection volume of 0.5 l and a split ratio of 10:1, and an injector temperature of 250°C, helium (99.999 percent) was employed as the carrier gas. The Elite-1 fused silica capillary column is built entirely of dimethyl polysiloxane (30 mm x 0.2 5mm ID x 1 Mdf)[17]. The oven's temperature was programmed to start at 110°C (isothermal for 2 minutes), rise by 10°C per minute to 200°C, then drop by 5°C per minute to 280°C, and finally finish at 280°C for 9 minutes. Mass spectra were gathered at a scan period of 0.5 seconds, scan energy of 70 eV, and fragment sizes ranging from 40 to 550 Da.

### *2.10 Identification of Phytochemical Constituents*

Using the National Institute of Standards and Technology's database, mass spectra from GC-MS were interpreted (NIST). The mass spectra of the unknown and known components kept in the NIST library were compared. The name, molecular weight, and structure of the SIX phytochemical constituents identified from the *Borassus flabellifer* sap were ascertained by GC-MS analysis and are presented in Table.1.

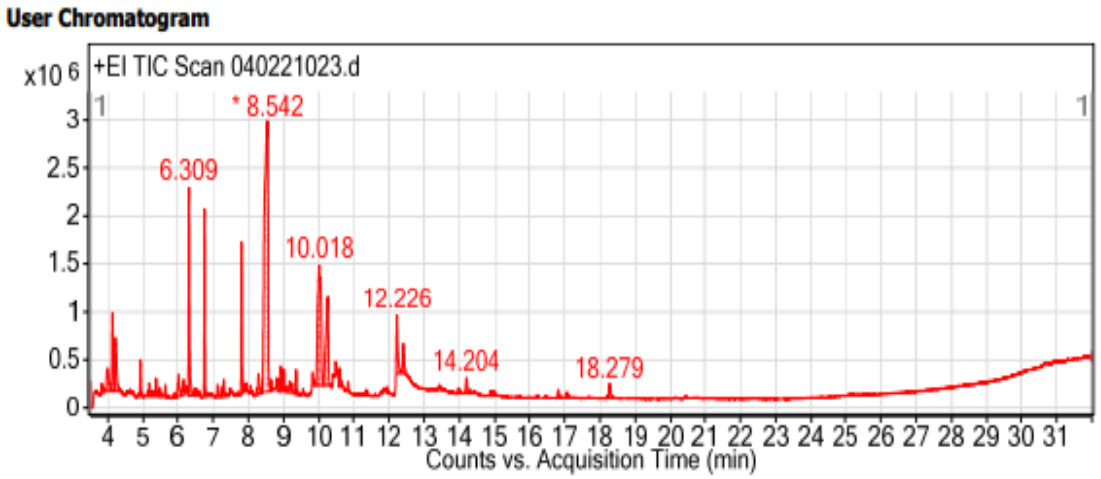



**Fig. 2. GCMS chromatogram of *Borassus flabellifer* plant extract**

**Table 1. Screened compounds from the sap of *Borassus flabellifer***

| GC-RT* | Compound name | PubChem ID | Docking Score (-Kcal/mol) |
|---|---|---|---|
| | | | 1O86 |
| 8.542 | Borinic acid, diethyl- | 543036 | -5.2 |
| 6.309 | 5-Hydroxymethylfurfural | 237332 | -4.7 |
| 10.018 | 3-Deoxy-d-mannoic lactone | 541561 | -5.7 |
| 12.226 | 9-Octadecenoic acid, (E)- | 637517 | -5.6 |
| 14.204 | D-Mannoundecane-1,2,3,4,5-pentaol | 552200 | -5.4 |
| 18.279 | 9,10-Anthracenedione, 1,8-dihydroxy-3-methyl- | 10208 | -8.6 |
| Native Ligand | Lisinopril | 5362119 | -7.3 |

***Selected major chemicals screened by using GC profile**

It was evident from table -1 that the sap from the chosen *Borassus flabellifer* contained a significant amount of phytochemicals. Through the use of GC-MS techniques, the following phytochemicals have been known to have been discovered: Borinic acid, diethyl, 5-Hydroxymethylfurfural, 3-Deoxy-d-mannoic lactone, 9-Octadecenoic acid, (E), D-Mannoundecane-1,2,3,4,5-pentaol, 9,10-Anthracenedione, 1,8-dihydroxy-3-methyl, and Among those, 9, 10, and Anthracenedione had high RT and a docking score of -8.6. The peaks of the evidence were clearly shown in Fig-2 with the identified GC-RT.

### *2.11 Potential Targets and Binding Site*

The 3D structures of angiotensin-converting enzyme (ACE) cleave angiotensin I to produce the active peptide vasoconstrictor hormone angiotensin II was retrieved from PDB database [18]. The active sites in these receptors were identified based on the crystalline structures' ligands. In addition, the interactions and the affinities between the phytochemical constituents and receptor were predicted by using Autodock Vina[19].

### *2.12 Ligand Generation*

The 3D structure of the identified phytocompounds (ligand molecules) from the *Borassus flabellifer* sap was obtained from the pubchem database. The obtained 3D SDF were submitted to an online SMILES converter and the structure file generated was converted to 3D PDB file format[20]. The obtained 3D PDB files of the ligands were utilized for further study.

### *2.13 Target and ligand optimization*

For docking analysis, target protein and ligand structure optimized using Discovery studio version 3.0 software. Optimized target and ligand structures had minimum energy and stable conformation.

### *2.14 Analysis of target protein active site*

Optimized target protein active site coordinates as well as the ligand in the original target protein grids, and these active binding sites of target protein were analyzed using the Drug Discovery Studio version 3.0 and 3DLigandSite virtual tools.

### *2.15 Molecular Docking*

By producing a separate score for each orientation, the molecular docking technique, which belongs to the field of molecular modeling, determines the optimal match orientation of a ligand (drug) to its target molecules (receptors). These points are referred to as docking points. AutoDock Vina explored the binding affinities between the receptors and ligands [21]. The grid map and grid size were calculated using auto grid to represent the protein binding size for docking. The grid size of 43x36x51 points in each dimension was set for 1O86. The spacing of 0.375Å was fixed between the grid points by Autogrid for the apoptotic regulatory proteins and Gasteiger charges were calculated using autodock tools. Assessment of docking (~ 100 times), size of population (150), energy evaluation (maximum number 250,000), generations (maximum number 27,000), rate of mutations (0.02), rate of cross-over (0.8), the value of elitism (1) and other parameters as default values were established using the autotors utility of the AutoDock tool to know the possible torsions of ligand molecules for docking. The docking pose with the better binding affinity score (kcal/mol) is ranked as top orientation for each ligand against each receptor and the binding interaction studies were analyzed. The docking interactions were analyzed using receptor-ligand interaction options in Discovery Studio v2.5 visualizer tool[22].

## 3. RESULT AND DISCUSSION

### *3.1 Phytochemical analysis*

Results of the phytochemical analysis of *Borassus flabellifer* are presented in Table 2 and show that plant extract possesses phytoconstituents like alkaloid, flavonoid, phenol, tannin, glycosides, saponin. Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes, and also act as anti-inflammatory agents (6). Phenolic compounds are essential as cellular support material because they form an integral part of cell wall structure by polymeric phenolics [23.] Bioactive polyphenols have attracted particular attention because they can protect the human body from oxidative stress, which may cause many diseases, including cancer, cardiovascular problems, and aging (8). Glycosides have been known to lower blood pressure [24]. Early life appears to offer a critical window of opportunity for launching interventions focused on preventing hypertension, as increasing evidence supports the supposition that hypertension can originate in early life[25]. A crucial enzyme, namely angiotensin-I converting the enzyme (ACE) plays a vital role in regulating blood pressure or hypertension [26]. It was known that the blood pressure occurred due to the conversion of angiotensin I into angiotensin II [27]. Therefore, to prevent high blood pressure, it is required to inhibit ACE activity [28]. In general, synthetic

medications for ACE inhibitors may cause adverse effects in people. Thus researchers have given natural compounds from medicinal plants priority since they are free of side effects, inexpensive, and have low doses that improve therapeutic outcomes.[29]. Congestive heart failure and hypertension are on the rise everywhere in the world. In 2000, nearly one billion cases of the adult population of the world had hypertension[30]. Plant-derived natural chemicals can be suitable as lead compounds to prevent the side effects of synthetic drug[31]. Increased blood pressure (BP) increases the risk of heart disease, stroke, obesity, kidney disease, and vision impairment. A significant incidence of hypertension has been associated with excessive salt consumption. A high dietary salt intake increases the risk of hypertension, which counteracts the BP-lowering effects of most antihypertensive medications. A diet high in fruits, vegetables, and low-fat dairy products have reportedly been shown to decrease blood pressure levels[32] significantly. The angiotensin-converting enzyme is the primary target in the therapy of hypertension. According to Victor H. Vázquez-Valadez [33](ACE) Angiotensin II, a powerful vasoconstrictor, is produced by this enzyme. Inhibiting ACE activity is thus one of the goals in treating hypertension. Therefore, this work aims to employ computational simulations to show that the heterocyclic compounds have a molecular affinity for ACE and can block ACE action, preventing the synthesis of the vasopressor angiotensin II. Figure 1 depicts the 3D structure of the target ACE protein. The use of medicinal plants extends back to antiquity.

Most currently accessible medicinal medications were developed directly or indirectly from plants, as they are a rich source of active ingredients. The therapeutic benefits of plant bioactive compounds have been lauded for their effectiveness in avoiding diabetes, cancer, inflammations, cardiovascular illnesses, etc[26]. This investigation aimed to uncover any putative ACE inhibitors that may be present in the palm sap. Molecular docking and virtual screening are efficient and cost-effective. Reliable methods for discovering a prospective druggable protein target and a new drug (lead molecule) using rational drug designing (RDD) or computer-aided drug design (CADD) The Docking program, Autodock vina was used to specify the binding surface of the receptors and the antihypertensive compounds in SDF format were used to dock within the binding pockets of ACE. The docking was carried out with a radius of 6.5 $A^0$ at the docking site. The docking interactions between the predicted binding site amino acids of ACE and the seven ligand molecules are shown in Table.1. The results of interactions between amino acids at the binding sites and the ligand molecules revealed the most important functional groups of the ligand molecules and the amino acids favoring the H bond and Hydrophobic interactions. It is observed that the highest docking interactions of -8.6 kcal/mol were exhibited by 9,10-Anthracenedione against ACE (Table 2).

**Table 2: Interactions of top scored compounds against two hypertensive enzyme targets**

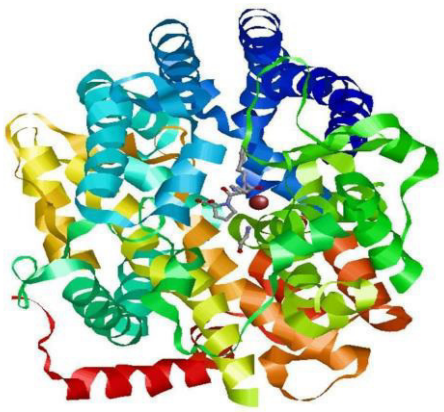
| Complex | Bonded | Non bonded | Docking score (kJ/mol) |
|---|---|---|---|
| 1O86-10208 | Ser289,His323,Trp473 | Phe282,Cys285,His449,Leu453,Leu465,Leu469 | -8.6 |

***Interactions of top-scored compounds against ACE***

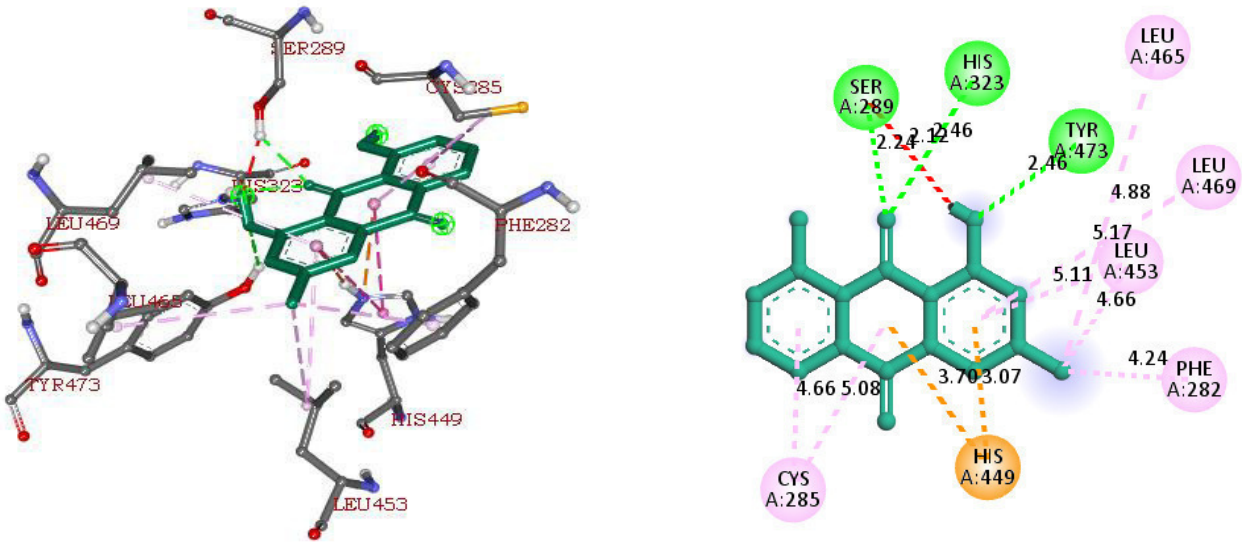
The top-scoring drugs against hypertensive enzyme targets were clearly displayed in Table 2. Here, the docking score of -8.6 allowed the identification of the bonded form (Ser289, His323, Trp473) and the non-bonded form (Phe282, Cys285, His449, Leu453, Leu465, Leu469).

### *3.2 Binding of 9,10-Anthracenedione with ACE*

In the ACE and lisinopril complex crystal structures, the binding interaction is favored by Ile456, Glu460, Thr461 and Met463 by forming H-bond interactions and the non-bonded interactions are favoured by Leu453, Lys457 and Leu465 with dock score of -7.3 kcal/mol. In contrast, the bioactive compounds from *Borassus flabellifer* sap exhibited the binding efficiencies ranging from-4.7 kcal/mol to -8.6 kcal/mol (Table 1). The best-docked compound 9,10-Anthracenedione demonstrated the binding score of -8.6 kcal/mol by forming the H-bond interactions with Ser289, His323, Trp473 and non-bonded interactions with Phe282, Cys285, His449, Leu453, Leu465, Leu469. Interestingly, the binding residues favoring H-bond residues are very different from the standard drug, the residues Leu453 and Leu465 were found to be conserved in favoring non-bonded interactions with the bioactive compound with relatively lower binding efficacy than the common drug.



***The protein structure of Human Angiotensin Converting Enzyme complexed with Lisinopril (PDB ID: 1O86)***




*A. 3D poses and 2D interaction plot for 1O86-10208 complex (-.8.6 Kcal/mol)*

**The docking complex of standard drug and 9,10-Anthracenedione against the ACE (-8.6 Kcal/mol)**

## 4. CONCLUSION

The study concludes that *Borassus flabellifer* sap's bioactive components are among the best non-cytotoxic natural extracts for treating hypertension. In a molecular docking analysis of bioactive compounds from *Borassus flabellifer* sap with enzyme targets related to hypertension, 9,10-anthracenedione was shown to be one of the intriguing substances. Through the docking and interaction pattern of ligands, the crucial residues of the enzyme's catalytic cavity or residues close to the active regions of these proteins were unusually susceptible to contact. As a result, 9,10-Anthracenedione is a viable bioactive compound identified in *Borassus flabellifer* sap and a contender for the development of nutraceuticals and functional foods, as well as an effective anti-hypertension medicine.

## 5. AUTHORS CONTRIBUTION STATEMENT

This study was done by M. Shobana under the guidance of Dr.S.Thilagavathi. we discussed the results and contributed to final manuscript.

## 6. CONFLICT OF INTEREST

Conflict of interest declared none.

## 7. REFERENCES


1. *Clark RD, Strizhev A, Leonard JM, Blake JF, Matthew JB. Consensus scoring for ligand/protein interactions. J Mol Graph Model. 2002;20(4):281-95. doi: 10.1016/s1093-3263(01)00125-5, PMID 11858637.*
2. *Gehlhaar DK, Verkhivker G, Rejto PA, Fogel DB, Fogel LJ. Docking conformationally flexible small molecules into a protein Binding Site through evolutionary programming. Proceedings of the fourth international conference on evolutionary programming 1995. p. 615-27.*
3. *Charifson PS, Corkery JJ, Murcko MA, Walters WP. Consensus scoring: A method for obtaining improved hit rates from docking databases of three-dimensional structures into proteins. J Med Chem. 1999;42(25):5100-9. doi: 10.1021/jm990352k, PMID 10602695.*
4. *Izzo JL, Black HR, Goodfriend TL. Hypertension primer: the essentials of high blood pressure. Lippincott Williams & Wilkins; 2003.*
5. *Natesh R, Schwager SL, Sturrock ED, Acharya KR. Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. Nature. 2003;421(6922):551-4..*
6. *Advanced Chemistry Development, Inc. ACD/ChemSketch freeware. Version 12. Toronto, ON, Canada: Advanced Chemistry Development, Inc. Available from: http://www.acdlabs.com.*
7. *Hebbar KB, Arivalagan M, Manikantan MR, Mathew AC, Thamban C, Thomas GV et al. Chowdappa Coconut inflorescence sap and its value addition as sugar–collection techniques, yield, properties and market perspective. Curr Sci. 2015:1411-7.*
8. *Fissore D, T. McCoy Freeze-drying and process analytical technology for pharmaceuticals. Front Chem. 2018;6:622.*
9. *Weininger D. Smiles, a chemical language and information system. Introduction to methodology and encoding rules. J Chem Inf Comput Sci. 1998;28:31-6.*
10. *Sari BL, Mun'im A, Yanuar A, Riadhi R. Screening of α-glucosidase inhibitors from terminalia Catappa L.*

*fruits using molecular docking method and in vitro test. Int J Pharm Pharm Sci. 2016;8(12):184-89. doi: 10.22159/ijpps.2016v8i12.14800.*

11. *Shobana M, inventor; Thilagavathi S , Method of Preparing nutrient Powder from Natural Extracts. Indin Patent 202041007640 A, 2020 Apr 4*
12. *C A, M S, Ts G. Structure-based docking studies toward exploring the potential anticancer activity of morin against non-melanoma skin cancer therapeutic drug targets. Asian J Pharm Clin Res. 2018;11(4):61-6. doi: 10.22159/ajpcr.2018.v11i4.23076.*
13. *Sussman JL, Lin D, Jiang J, Manning NO, Prilusky J, Ritter O, et al. Protein data bank (PDB): database of three-dimensional structural information of biological macromolecules. Acta Crystallogr D Biol Crystallogr. 1998;54(6 Pt 1):1078-84. doi: 10.1107/s0907444998009378, PMID 10089483.*
14. *Rarey M, Kramer B, Lengauer T, Klebe G. A fast flexible docking method using an incremental construction algorithm. J Mol Biol. 1996;261(3):470-89. doi: 10.1006/jmbi.1996.0477, PMID 8780787.*
15. *Gnanendra S, Anusuya S, Natarajan J. Molecular modeling and active site analysis of SdiA homolog, a putative quorum sensor for Salmonella typhimurium pathogenecity reveals specific binding patterns of AHL transcriptional regulators. J Mol Model. 2012;18(10):4709-19. doi: 10.1007/s00894-012-1469-1, PMID 22660944.*
16. *Stierand K, Maass PC, Rarey M. Molecular complexes at a glance: automated generation of two-dimensional complex diagrams. Bioinformatics. 2006;22(14):1710-6. doi: 10.1093/bioinformatics/btl150, PMID 16632493.*
17. *Tain Y-L, Hsu CN. Novel insights on dietary polyphenols for prevention in early-life origins of hypertension: a review focusing on preclinical animal models. Int J Mol Sci. 2022. Jun 14;23(12):23(12):6620. doi: 10.3390/ijms23126620, PMID 35743061.*
18. *Kumar R, Sharma R, Bairwa K, Roy RK, Kumar A, Baruwa A. Modern Development in ACE inhibitors. Pharm Lett. 2010;2(3):388-419.*
19. *Natesh R, Schwager SLU, Sturrock ED, Acharya KR. Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. Nature. 2003;421(6922):551-4. doi: 10.1038/nature01370, PMID 12540854.*
20. *Cushman DW, Ondetti MA. Design of angiotensin converting enzyme inhibitors. Nat Med. 1999;5(10):1110-3. doi: 10.1038/13423, PMID 10502801.*
21. *Muthia R, Suganda AG, Sukandar EY. Angiotensin-I converting enzyme (ACE) inhibitory activity of several Indonesian medicinal plants. Res J Pharm Biol Chem Sci. 2017 [Suppl];8(1S):192-9.*
22. *Jandeleit-Dahm K, Cooper ME. Hypertension and diabetes: role of the renin-angiotensin system. Endocrinol Metab Clin North Am. 2006;35(3):469-90. vii. doi: 10.1016/j.ecl.2006.06.007, PMID , Google Scholar.*
23. *Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005;365(9455):217-23. doi: 10.1016/S0140-6736(05)17741-1, PMID 15652604, Google Scholar.*
24. *White WB, Prisant LM, Wright JT Jr. Management of patients with hypertension and diabetes mellitus: advances in the evidence for intensive treatment. Am J Med. 2000;108(3):238-45. doi: 10.1016/s0002-9343(99)00444-1, PMID 10723978.*
25. *Park HA. Fruit intake to prevent and control hypertension and diabetes. Korean J Fam Med. 2021 Jan;42(1):9-16. doi: 10.4082/kjfm.20.0225, PMID 33524250.*
26. *Vázquez-Valadez VH, Abrego VH, Martínez PA, Torres G, Zúñiga O, Escutia D, et al. Docking studies of Methylthiomorpholin phenols (LQM300 series) with angiotensin-converting enzyme (ACE). Open Med Chem J. 2013;7:30-8. doi: 10.2174/1874104501307010030, PMID 24319502.*
27. *Walia A, Gupta AK, Sharma V. Role of bioactive compounds in human health. Acta Sci Med Sci. 2019;3(9):25-33.*
28. *Chandra Mohan S, Dinakar S, Anand T, Elayaraja R, SathiyaPriya B. Phytochemical, GC-MS analysis and antibacterial activity of a medicinal plant Acaly phaindica. Int J Pharm TechnolRes. 2012;4:1050-4.*
29. *Gothandam KM, Aishwarya R, Karthikeyan S. Preliminary screening of antimicrobial properties of few medicinal plants. J Phytol. 2010;2:1-6.*
30. *Siddiqui AA, Ali M. Practical pharmaceutical chemistry. 1stEdn, CBS Publishers and distributors, New Delhi, 126-31, 1997.*
31. *Selvakumarsivagnannam, Aravindkumar. Preliminary phytochemical analysis of Tabernaemontana alternifolia. Int J Pharm Biol Sci. 2014;5(2): (B) 283-287.*
32. *Evans WC. Trease and Evan's pharmacognosy. 5thEdn, Haarcourt brace and company. Vol. 336; 2002.*
33. *Frankel E. Nutritional benefits of flavonoids, International conference on food factors: chemistry and cancer prevention, Hamamatsu, Japan. Admin S. 1995:C-2.*
34. *Gupta VK, Singh GD, Singh S, Kaul A. Medicinal plants: phytochemistry, Pharmacolgoy and therapeutics. Delhi: Daya Publishing House; 2010.*