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Prediction of Anti-Hypertensive Potential of Siddha Formulation – *Sagalanoi Chooranam* against Angiotensin-Converting Enzyme (ACE): A Computational Approach

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Abstract: Hypertension, one of the top non-communicable diseases in the world, is a significant risk factor for the nephritic syndrome, coronary disease, heart failure, stroke and a host of other cardiovascular conditions which can be well-treated or controlled by Angiotensin Converting Enzyme (ACE) inhibitors. About 20-97% of hypertensive patients taking anti-hypertensive drugs experience side effects. Siddha's literature has numerous drugs capable of lowering hypertension which could be used as an alternative or supportive to anti-hypertensive drugs. Since hypertensive patients need medication throughout their lifetime to maintain their blood pressure, showing concern in choosing their regular medicines and their safety is mandatory. The present study investigatedq the anti-hypertensive activity of *Sagalanoi Chooranam* using computational analysis. The structure of the target protein (Angiotensin Converting Enzyme – PDB id 1O86) and selected ligands from the ingredients of *Sagalanoi Chooranam* were collected from databases. Docking studies were performed using Auto Dock Software Version 4. The performed docking studies showed satisfying results with the binding affinity energy of -7.80, -6.25, -6.19 and -6.00 kcal/mol for Glycyrehhtic Acid, biotin, Isoginkgetin and β-Caryophyllene, respectively against a target protein, Angiotensin Converting Enzyme. These bioactive compounds show better results than the standard drug, Captopril. On this basis, it was revealed that the phytochemicals of the ingredients of Siddha formulation *Sagalanoi Chooranam* were expected to show anti-hypertensive activity by inhibiting Angiotensin Converting Enzyme.

Keywords: Auto Dock, Angiotensin Converting Enzyme, Hypertension, *Sagalanoi Chooranam*, Siddha

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Introduction

Hypertension is one of the top most prevalent non-communicable diseases in the world. In 2019, approximately one billion people with hypertension were identified from low- and middle-income countries. A global network of physicians and researchers study found that the burden of hypertension has shifted from wealthy countries to low – and middle-income countries in the past three decades. Hypertension is the most significant stroke risk factor and one of India's top three coronary risk factors (Gupta, 2018). Even though it is one of the single leading contributors to all-cause death and disability universally, as well as in India. It is the most preventable risk factor for cardiovascular disease (CVD) when diagnosed and managed earlier.

Hypertension technically means systemic arterial hypertension characterized by a persistent increase or high blood pressure in systemic arteries. Blood pressure (BP) is usually represented as the systolic pressure over the diastolic pressure and is measured in millimetres of mercury (mmHg) units. Effective prevention and management of hypertension are crucial in decreasing the disease burden and promoting the longevity of human life. In managing hypertension, it is important to assess predicted atherosclerotic CVD (ASCVD) risk more than the measurement of BP alone, as patients with high CVD risk get the utmost benefit from BP-reducing treatment. About 20-97% of hypertensive patients taking anti-hypertensive drugs experience side effects. Few prior research works suggest that drug side effects are the main determinant in a decreased adherence rate to anti-hypertensive drugs (Bardage and Isacson, 2000).

To overcome this challenge, people need new drug entities with fewer or no side effects with high palatability. In recent times, herbal medicines are gaining more attention and interest from the public, attributed to several factors, including the high cost and side effects of modern drugs (Morgado *et al*., 2010). Traditional medicine systems possess many formulations with herbs and their parts. Siddha, one of the AYUSH systems of medicine, has been widely practised in regions of South India for thousands of years. Siddha's literature has numerous drugs capable of lowering hypertension which could be used as an alternative or supportive to anti-hypertensive drugs (Bandaranayake, 2006). Since hypertensive patients need medication throughout their lifetime to maintain their blood pressure, showing concern in choosing their regular medicines and their safety is mandatory. One such formulation is *Sagalanoi Chooranam*, which comprises six medicinal herbs mentioned in Siddha literature to be used clinically for diseases like hypertension, head diseases, ear diseases, renal calculi, insomnia, etc.

A significant alternate approach for screening millions of bio-active compounds is Virtual screening, which can be achieved within a few days. Molecular docking is one of the most pragmatic virtual screening methods. This method could predict both the binding affinity between ligand and protein and the structure of protein–ligand complex, which is useful information for lead optimization (Lavecchia and Giovanni, 2013). In the present study, the active compounds of ingredients of *Sagalanoi Chooranam* were screened, and they were done for docking analysis against the target protein, Angiotensin-Converting Enzyme (ACE), using Auto Dock software.

Materials and Methods

The objective of the present study was to predict the efficacy of the lead candidates to bind with these core bioactive amino acid residues, GLU162, ALA354, GLN281, HIS383, GLU384, HIS353, HIS387, GLU411, LYS511, HIS513, TYR520, TYR523 which facilitates the enzymatic action of the ACE that has a major role in controlling blood pressure. ACE succeeds in its activity by converting angiotensin I into angiotensin II. The converted angiotensin-II can increase blood pressure by constricting the blood vessels. Hence, inhibiting the action of ACE by occupying the above-mentioned amino acids will reduce blood

pressure by depleting the release of Angiotensin II. Lead molecules or Phytochemicals that can inhibit the enzyme ACE will be considered to have anti-hypertensive activity, and hence, it could be used in managing hypertension.

Trial Drug – Sagalanoi Chooranam:

The trial drug, *Sagalanoi Chooranam* consists of six herbal ingredients namely, *Seeragam* (*Cuminum cyminum* L.), *Athimathuram* (*Glycyrrhiza glabra* L.), *Mathanakaama Poo* (*Cycas circinalis* L.), *Karunseeragam* (*Nigella sativa* L.), *Lavangam* (*Syzygium aromaticum* L. Merr. & Perry.) and *Kothumalli* (*Coriandrum sativum* L.). As per Siddha concept, vitiated *Pitham* (one of the three bodily humors) causes hypertension in an individual. Majority of these ingredients are meant to be used in the reduction of *Pitham*. Hence, the hypothesis is that this formulation may work as an anti-hypertensive drug. Yet, this needs to be verified using scientific research works.

Protein selection and Preparation:

Angiotensin-Converting Enzyme (ACE) has been the target of hypertension control since the 1970s. ACE cleaves Angiotensin-I hormone into the vasoconstricting Angiotensin-II which causes a cascade of hormonal reactions which is part of the body's harmful phase of RAAS, which ultimately leads to an increase in the body's blood pressure. The 3D structure of the target protein, ACE (Fig. 1) was retrieved from Protein Data Bank (https://www.rcsb.org/) (PDB ID - 1O86) and subjected to protein cleaning prior to docking. After the protein clean-up process was done, the essential missing hydrogen atom was added.

Ligand selection and Preparation:

After a thorough literature survey, 10 bio-active phytochemicals namely Glycyrrhizic acid, Carvacrol, cuminaldehyde, Linalool, coumaric acid, β-pinene, α-Thujone, β-caryophyllene, Isoginkgetin, Bilobetin, were screened and selected as lead compounds from the ingredients of the trial drug (Table 2). The three-dimensional structure of selected phytochemicals was built using Chem Draw prof online tool version 12.0. which is depicted in Figure 2. Ligands prepared through geometry optimization method (MMFF94). Captopril is a standard used as an anti-hypertensive drug, which is taken into consideration for docking analysis in order to compare the results of the phytochemicals of the trial drug.

Docking Analysis:

Different orientation of the lead molecules with respect to the target protein was evaluated by the Autodock version 4 program and the best dock pose was selected based on the interaction study analysis. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking analyses were carried out for retrieved Phyto-components against target protein ACE. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock - 4 virtual screening tools. Affinity (grid) maps of 60×60×60 Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method. The initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied (Batiha *et al*., 2020).

The 10 selected ligands and the standard drug, Captopril's structures were retrieved along with the structure of the target protein, ACE. They were analyzed using Auto Dock version 4 to

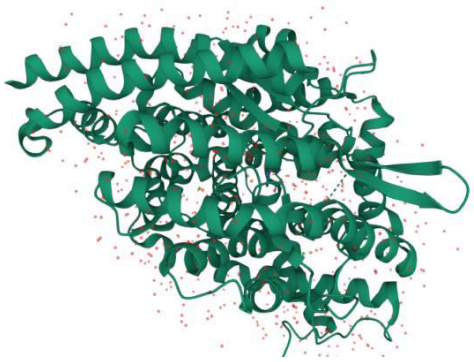

Fig. 1: Crystalline Receptor Structure of Human Angiotensin-converting enzyme (PDB ID-1O86).

Table 1: List of Phyto-components Selected for Docking

S. No.	Ingredients of *Sagalanoi Chooranam*	Selected Phyto-Components
1	*Glycyrrhiza glabra* L.	• Glycyrrhizic acid
2	*Cuminum cyminum* L.	• Carvacrol • Cuminaldehyde • Linalool • Coumaric acid
3	*Nigella sativa* L.	• β-pinene
4	*Anethum sowa* L.	• α-Thujone
5	*Syzygium aromaticum* L. Merr. & Perry.	• β-caryophyllene
6	*Cycas circinalis* L.	• Isoginkgetin • Bilobetin

Table 2: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Glycyrrhizic acid	470.7 g/mol	$C_{30}H_{46}O_4$	2	4	1
Carvacrol	150.221 g/mol	$C_{10}H_{14}O$	1	1	1
Coumaric Acid	164.16 g/mol	$C_9H_8O_3$	2	3	2
Cuminaldehyde	148.205 g/mol	$C_{10}H_{12}O$	0	1	2
Linalool	154.25 g/mol	$C_{10}H_{18}O$	1	1	4
β Pinene	136.23 g/mol	$C_{10}H_{16}$	0	0	0
α-Thujone	152.23 g/mol	$C_{10}H_{16}O$	0	1	1
β Caryophyllene	204.35 g/mol	$C_{15}H_{24}$	0	0	0
Captopril	217.29 g/mol	$C_9H_{15}NO_3S$	2	4	3
Isoginkgetin	566.5 g/mol	$C_{32}H_{22}O_{10}$	4	10	5
Bilobetin	552.5 g/mol	$C_{31}H_{20}O_{10}$	5	10	4

Table 3: Amino acid Residue Interaction of Lead and Standard against Crystal structure of Angiotensin converting enzyme

S. No	Compound	Inter-actions	Amino-acid Residues														
1	Linalool	4	162 GLU	352 CYS	353 HIS	354 ALA	369 GLN	370 CYS	377 ASP	380 VAL	384 GLU						
2	Beta-Pinene	5	281 GLN	457 PHE	511 LYS	513 HIS	520 TYR	523 TYR	527 PHE								
3	Alpha-Thujone	3	162 GLU	353 HIS	354 ALA	369 GLN	370 CYS	372 THR	377 ASP	380 VAL							
4	Beta-caryophyllene	6	353 HIS	354 ALA	384 GLU	457 PHE	513 HIS	520 TYR	523 TYR								
5	Carvacrol acetate	9	281 GLN	353 HIS	383 HIS	384 GLU	387 HIS	411 GLU	415 ASP	457 PHE	513 HIS	520 TYR	523 TYR	527 PHE			
6	Glycyrrhizic acid	3	70 ASN	143 GLU	355 SER	357 TRP	387 HIS	391 PHE	410 HIS	411 GLU	512 PHE	516 SER	518 VAL	522 ARG	523 TYR		
7	Coumaric Acid	2	162 GLU	354 ALA	369 GLN	370 CYS	377 ASP										
8	Cuminaldehyde	7	281 GLN	383 HIS	411 GLU	415 ASP	457 PHE	511 LYS	513 HIS	520 TYR	523 TYR	526 SER	527 PHE				
9	Isoginkgetin	7	162 GLU	353 HIS	380 VAL	383 HIS	384 GLU	387 HIS	410 HIS	411 GLU	523 TYR						
10	Bilobetin	9	162 GLU	279 TRP	281 GLN	353 HIS	355 SER	380 VAL	383 HIS	384 GLU	387 HIS	411 GLU	512 PHE	513 HIS	518 VAL	523 TYR	2000 GLY
11	Captopril	8	281 GLN	353 HIS	380 VAL	383 HIS	384 GLU	457 PHE	511 LYS	513 HIS	520 TYR	523 TYR	2000 GLY				

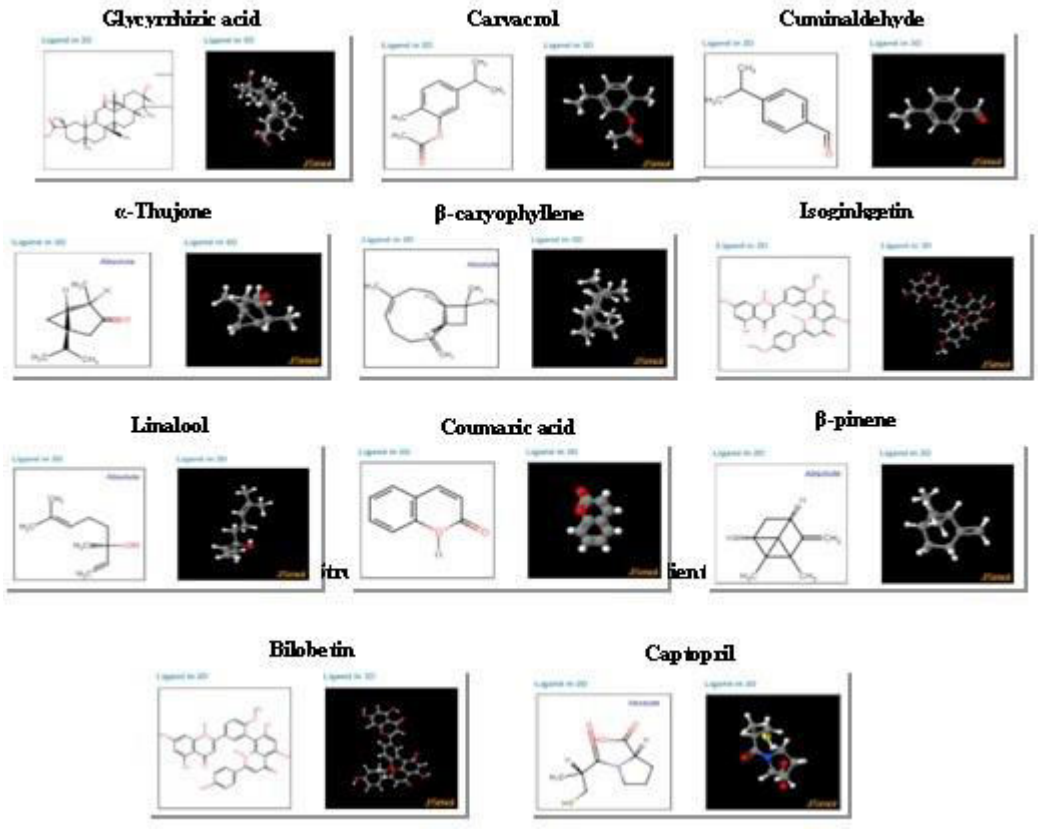

Fig. 2: 2D and 3D Structure of Selected Ligands of ingredients of *Sagalanoi Chooranam.*

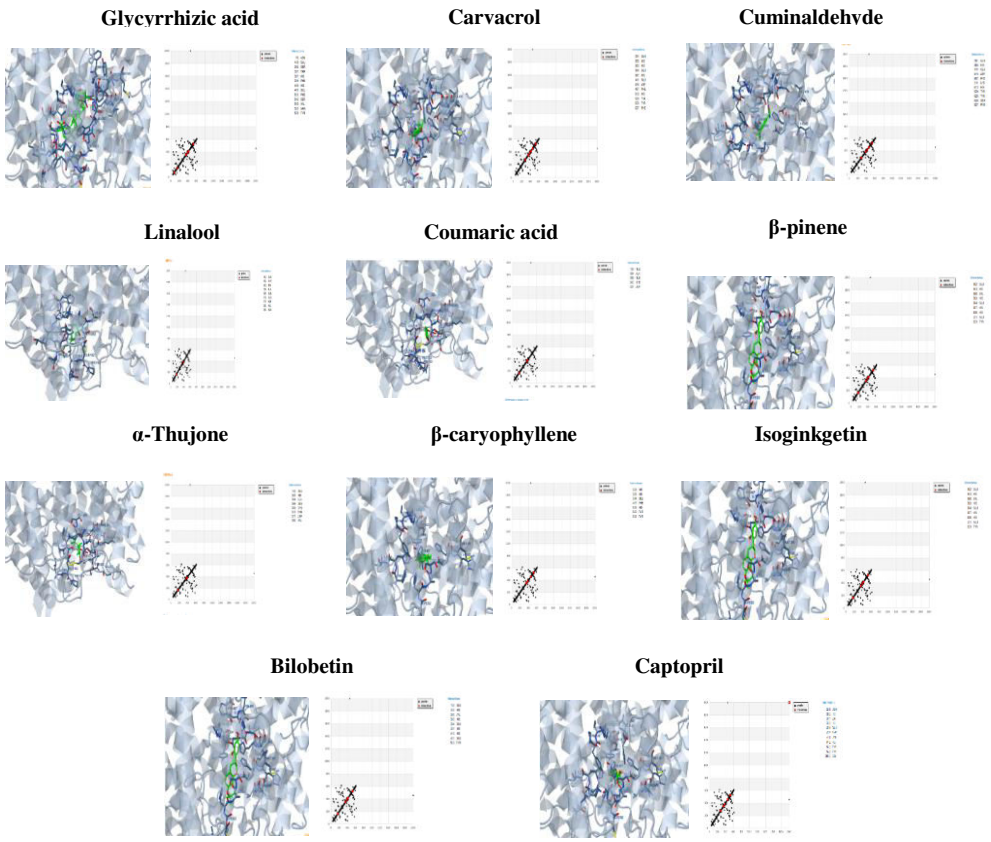

Fig. 3: Representing best docking pose and hydrogen bond plotting with core amino acid analysis of lead molecules against Crystal Structure of Angiotensin-Converting Enzyme

Table 4: Summary of the molecular docking studies of compounds against Angiotensin-converting enzyme (1O86)

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Glycyrrhizic acid	-7.80 kcal/mol	1.90 uM	-0.26 kcal/mol	-8.40 kcal/mol	859.828
Carvacrol	-4.67 kcal/mol	374.84 uM	-0.19 kcal/mol	-5.59 kcal/mol	559.801
Coumaric Acid	-4.08 kcal/mol	1.03 mM	-0.12 kcal/mol	-4.08 kcal/mol	451.406
Cuminaldehyde	-4.15 kcal/mol	914.00 uM	-0.01 kcal/mol	-4.74 kcal/mol	452.775
Linalool	-4.52 kcal/mol	484.36 uM	-0.19 kcal/mol	-5.98 kcal/mol	506.988
β Pinene	-4.81 kcal/mol	298.44 uM	-0.01 kcal/mol	-4.81 kcal/mol	420.523
α-Thujone	-4.47 kcal/mol	527.15 uM	-0.03 kcal/mol	-4.77 kcal/mol	447.393
β Caryophyllene	-6.00 kcal/mol	40.20 uM	-0.01 kcal/mol	-6.00 kcal/mol	569.496
Isoginkgetin	-6.19 kcal/mol	29.05 uM	-0.42 kcal/mol	-7.05 kcal/mol	805.029
Bilobetin	-6.25 kcal/mol	26.11 uM	-0.53 kcal/mol	-6.93 kcal/mol	966.705
Captopril	-5.09 kcal/mol	186.95 uM	-0.61 kcal/mol	-5.13 kcal/mol	504.357

assess the binding affinity between the ligands and the target protein.

Results and Discussion

A total of 10 bioactive lead compounds were retrieved from the herbs present in the Siddha formulation *Sagalanoi Chooranam* as mentioned in Table 1 (Matter and Sotriffer, 2011). The ligand properties were verified and noted before docking analysis (Table 2). The docking analysis of the lead compounds against the target protein shows that the phytochemicals such as Beta-Pinene, Beta-caryophyllene, Carvacrol, Cuminaldehyde, Isoginkgetin and Bilobetin possess 5 to 9 interactions with the core amino acid residues and present on the target ACE (Wong, 2016). The other selected compounds such as Linalool, Alpha-Thujone, Glycyrrhizic acid and Coumaric Acid classified as second with 2-4 interactions with the active site of ACE when compared to the standard drug Captopril which reveals 8 interactions over the target enzyme (Table 3) (Saleh, 2010).

The docking analysis was done with proper documentation of 3D images of best docking poses during molecular interactions and hydrogen bond plotting with core amino acid analysis of lead molecules against Crystal Structure of Angiotensin-Converting Enzyme (Fig. 3) (Moawad *et al*., 2010).

The binding free energy during docking analysis of the phytochemicals such as Glycyrrhizic acid, Carvacrol, Coumaric Acid, Cuminaldehyde, Linalool, β Pinene, α-Thujone, β Caryophyllene, Isoginkgetin, Bilobetin and the standard drug, Captopril was found to be -7.80, -4.67, -4.08, -4.15, -4.52, -4.81, -4.47, -6.00, -6.19, -6.25, -5.09 kcal/mol, respectively (Bikadi and Hazai, 2009). These results are summarized including the details of the Inhibition constant, Electrostatic energy, intermolecular energy and total interaction surface (Table 4).

Conclusion

The present study of molecular docking analysis of the lead compounds from the ingredients of *Sagalanoi Chooranam* against the target protein, ACE was found to reveal significant results exhibiting anti-hypertensive activity. The binding free energy during docking analysis of the phytochemicals such as Glycyrrhizic acid, Bilobetin, Isoginkgetin and β Caryophyllene were found to have a high binding affinity with low binding energy than the standard drug, Captopril which was found to be -7.80, -6.25, -6.19, -6.00, and -5.09 kcal/mol, respectively. Hence, it is concluded that the phyto-chemicals present in the formulation *Sagalanoi Chooranam* may possess significant anti-hypertensive activity which needs to be confirmed through wet lab experiments.

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References

Bandaranayake WM. (2006) Quality control, screening, toxicity, and regulation of herbal drugs. In: Modern Phytomedicine. Turning Medicinal Plants into Drugs, (eds.) Ahmad I., Aqil F. and Owais M., Weinheim: Wiley-VCHGmbH & Co. KGaA, pp. 25-57.

Bardage C and Isacson DG. (2000) Self-reported side-effects of antihypertensive drugs: an epidemiological study on prevalence and impact on health-state utility. Blood Press. 9(6): 328-334.

Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH and Rashwan EK. (2020) *Syzygium aromaticum* L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules 10(2): 202.

Bikadi Z and Hazai E. (2009) Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminf. 1: 15.

Gupta R. (2018) Recent trends in hypertension epidemiology in India. In: CSI Textbook of Cardiology, (ed.) Deb P.K., Jaypee Brothers Medical Publishers, New Delhi.

Lavecchia A and Di Giovanni C. (2013) Virtual screening strategies in drug discovery: a critical review. Curr Med Chem. 20(23): 2839-2860.

Matter H and Sotriffer C. (2011) Applications and success stories in virtual screening. In: Virtual screening: principles, challenges, and practical guidelines, (ed.). Sotriffer C., Wiley-VCH Verlag GmbH & Co. KGaA, WeinheimGermany, pp. 319-358.

Moawad A, Hetta M, Zjawiony JK, Jacob MR, Hifnawy M, Marais JP and Ferreira D. (2010) Phytochemical investigation of *Cycas circinalis* and *Cycas revoluta* leaflets: moderately active antibacterial biflavonoids. Planta Med. 76(8): 796-802.

Morgado M, Rolo S, Macedo AF, Pereira L and Castelo-Branco M. (2010) Predictors of uncontrolled hypertension and antihypertensive medication nonadherence. J Cardiovasc Dis Res. (4): 196-202.

Saleh MM. (2010) Chemical constitutents of essential oil from *Anethum sowa* growing in Bangladesh. Bangladesh J Sci Ind Res. 45(2): 173-176.

Wong MKS. (2015) Angiotensin Converting Enzymes. Handbook of Hormones. doi: 10.1016/B978-0-12-801028-0.00254-3.