Original Research Article

ANTIHYPERTENSIVE AND *IN SILICO* DOCKING STUDIES OF PHYTOCONSTITUENTS ISOLATED FROM *SYZYGIUM ALTERNIFOLIUM* BARK

ABSTRACT

Aims: Pharmacological examination was done for *Syzygium alternifolium* bark methanol extract (MESA) for its antihypertensive activity, ACE inhibition by *in vitro* and *in vivo* studies, antioxidant activity *via* radical rummaging activity. Albino Wistar rodents were allowed to treat with Dexamethasone (30 µg/kg/day *s.c*) or saline for about 14 days. Methanolic extract of *Syzygium alternifolium* (300 mg/kg b.w., *p.o.*) is administered from day 8 to 14 day of study to treatment group. Chronic fructose treatment in rodents has over and over been displayed to raise circulatory strain in relationship with insulin obstruction. MESA (300 mg/kg b.w, *p.o*) capable of forestall the foundation of hypertension by diminishing the raised circulatory levels i.e., blood pressure. The decrease in the blood pressure is accredited to the restraint of ACE. The primer phytochemical examination proposes that the MESA has alkaloids, flavonoids, glycosides, steroids, sugars, proteins additionally tannins. MESA showed 1, 1-diphenyl-2-picrylhydrazyl interrupting radical chain reactions with IC_{50} worth of 12.34 µg/ml just as superoxide particle extinguishing capacity with IC_{50} worth of 21 µg/ml. MESA shows antihypertensive movement by restraining angiotensin changing over catalyst and interrupted free radical reactions i.e., antioxidant property. These discoveries uncover the existence of expected dynamic constituents of MESA. Understanding the molecular method of activity of natural product is a vital stage for creating drugs from them. The docking recreations of the multitude of mixtures were performed utilizing Maestro, implemented from mCule software. The constituents have good binding ability with ACE 1 inhibitor, Calcium channel blocker, and Renin inhibitor proteins and Ramachandran plot is analyzed. The compounds from *Syzygium alternifolium* bark have shown antihypertensive movement in contrast with standard medication Amlodipine.

Keywords: *Syzygium alternifolium* Bark, Dexamethasone, Hypertension, Fructose, Docking studies.

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1. INTRODUCTION

Hypertension is cardiovascular sickness, some place in raised blood vessel stress causes obsessive differences in the hypertrophy and vasculature of left heart ventricle. Hypertension is viewed as a condition of oxidative pressure that can add to increase of atherosclerosis and other hypertension prompted organ mutilation. Angiotensin changing over protein (Angiotensin converting enzyme), results angiotensin II from angiotensin I and bradykinin (a hypotensive peptide) to inactive components. High ACE action prompts expanded conversion of the angiotensin II and hypertension. Consequently, improvement of agents that obstruct the change of angiotensin I - angiotensin II, and bradykinin to latent parts started as a remedial methodology to treat hypertension. Inhibitors of ACE are important in the treatment towards hypertension & the maintenance of electrolyte balance. Captopril, besides is known to have negative effects when used for a long time. Because ROS-induced free radical stress in cardiac and vascular myocytes has proved to produce cardiovascular tissue injury and maintain vascular wall homeostasis, an equilibrium between ROS, endogenous transmitters angiotensin II & nitric oxide is critical. Hypertension aroused by chronically elevated levels of angiotensin II is interceded by superoxide anions partly, as has been well documented [1].

In our investigation, dexamethasone and fructose were employed to persuade hypertension, while amlodipine applied as reference medication. Chronic dexamethasone use results in very regular manifestation *i.e.*, hypertension. Specific mechanism is still to be discovered. Dexamethasone-induced hypertension was linked towards changes in numerous pathophysiological systems that affect blood pressure, including plasma volume, sympathetic activity, the renin-angiotensin-aldosterone system, vasodepressor & vasopressor systems. [2]. In rats, increased intake of either sucrose or glucose accelerated the occurrence of voluntary hypertension or salt hypertension. The exact mechanism for fructose inducing hypertension is unknown. The vasoconstrictive response is associated by Increase-fructose diet is not related to high activity of RAA pathway, according to evidence [3]. Insulin low sensitivity & hyperinsulinemia were thought to have part in the etiology of fructose-induced hypertension [4]. *Syzgium alternfolium* (Wight) Walp. (*Myrtaceae*), sometimes known as "Mogi," is a moderate-sized deciduous endemic tree found in abundance in the Seshachalam Hill ranges of the Southern Eastern Ghats, particularly in the Tirumala Hills. Alkaloids, flavonoids, indoles, steroids, carbohydrates, phenols, proteins, lignins, saponins, triterpenoids (friedelane, friedelinol) and stigmasterol can all be identified in bark of stem. The stem & fruits are long been utilized for diabetes treatment in the traditional medical system [5].

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Presently research for, molecular docking of ACE, renin and calcium channel inhibitors were performed using diverse computational tools, in perspect to find the optimum inhibitor, which ultimately would provide the basis towards designing drugs against hypertension. The goal of our research is to perform *in vitro, in vivo* antihypertensive activity, antioxidant activity and *In silico* analysis by docking and Ramachandran plot

2. MATERIALS AND METHODS

2.1 Plant material collection and drying

The bark of the *Syzygium alternifolium* was collected in Andhra Pradesh, India. In the month of July 2011, pharmacognist discovered this item. Plant samples were prepared and sent to Dr. K. Madhava Chetty, botanist at S.V. University in Tirupati, for certification, which he verified. To eliminate adherent particles and trash, the entire plant was thoroughly cleaned with water and dried in the sun. Dried material of plant was allowed to ground with a pulverizer and sieved # 20 before being stored in an airtight container until castoff.

2.2 Preparation of *Syzygium alternifolium* methanolic extract (MESA)

Simple distillation was used to extract the powdered plant material from 500 ml of methanol, and the plant material was suspended in a round bottomed flask containing the extraction solvent. After that, a condenser was added, and the flask was heated, allowing active extract components to enter the fluid. Source was filtered at the end of the extraction procedure. The surplus was evaporated, and the extracts were maintained in desiccators to remove any remaining moisture before being stored in airtight ampoules for future use.

2.3 Preliminary Phytochemical Investigation of Plant

The plant is a biosynthetic laboratory, producing a variety of compounds such as glycosides, alkaloids, volatile oils, tannins, and other physiological and medicinal substances in addition to chemical molecules such as carbohydrates, protein, and lipids. MESA was examined for the presence of a number of different chemicals.

2.4 Acute toxicity testing

Toxicity tests were brought out in order to determine the effects of the *Syzygium alternifolium* extract. The OECD 425 guidelines were followed when conducting acute toxicity tests. The first test, i.e., sequential test, is a limit test in which a maximum of five animals are used. A 2000 mg/kg test dose or, in rare cases, 5000 mg/kg may be utilized.

2.5 Experimental protocol

Albino research in Hyderabad provided Wistar albino rats (about 170-200 grammes). The current study was conducted in the Gokaraju Rangaraju College of Pharmacy's CPCSEA-

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approved animal house in Bachupally, Hyderabad, India. 1175/PO/ERe/S/08/CPCSEA (Reg. No. 1175/PO/ERe/S/08/CPCSEA). The animals were kept in poly acrylic cages with a 12-hour light/12-hour dark cycle, with no more than six animals per cage. Rats have unrestricted access to a conventional food and unlimited water. The albino mice were allowed to spend eight days in the preclinical laboratory environment before the experiment began. The albino mice were cared for and maintained according to the Committee for the Pursuit of Excellence's approved requirements.

2.6 *In Vitro* Antioxidant Activity

2.6.1 DPPH radical scavenging activity

In the appearance of DPPH stable radical, the hydrogen-donating capacity of MESA was tested. 2.5 microliter of test solution containing various amounts of MESA were added to one mL of 0.3 mM DPPH and permitted to react at normal temperature. Following 30 minutes of development, at the 517nm absorbance measurement is done. Methanol (1.0 mL) served as the blank, DPPH solution (1.0 mL, 0.3 mM) and methanol (2.5 mL) are the negative control. As the gold standard, ascorbic acid is taken [6].

2.6.2 Superoxide radical scavenging assay

1.4 milliliter of 50 mM potassium dihydrogen phosphate-potassium hydroxide, pH 7.4, including 1 mM EDTA, 0.5 mL of 100 L hypoxanthine, and 0.5 mL of 100 L NBT were used to make the reaction mixture of 3 milliliter in each tube. The reaction was begun by mixing 0.066 units of xanthine oxidase in 100 mL of phosphate buffer, pH 7.4, with 0.5 mL of MESA extract in saline. At 560 nm, NBT decrease was determined using a spectrophotometric technique. Gallic acid is taken as a reference, and the same approach was applied to assess MERB. The data were presented as a percentage of NBT inhibition [7].

2.7 Antihypertensive activity

Antihypertensive activity was evaluated *in vitro* using angiotensin converting enzyme inhibitor and two *in vivo* models, such as dexamethasone induced and fructose induced hypertension.

2.7.1 *In vitro* evaluation of ACE-inhibitory activity

The spectrophotometric test was used to assess ACE inhibitory activity in vitro. Rabbit lung provides the substrate, hippuryl histidyl-leucine (HHL), and angiotensin converting enzyme (ACE). Testing solutions (40 µL) were incubated for 30 minutes at 37 °C with 100 microliter of 0.1 M borate buffer (pH 8.3) containing 5 mM HHL and 0.3 M NaCl, as well as 20 microliter of ACE (2 mU), before being stopped, added with 150 microliter of 1 M HCl. The produced hippuric acid was extracted with 1000 µlitres of ethyl acetate, centrifuged at 1500

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rpm for 10 minutes, and 750 µlitres of the organic phase were allowed to evaporate. The residue added to 800 litres of distilled water so that it dissolves and the absorbance at 228 nm was measured. Each trial was carried out in triplicate. Inhibitory activity refers as protein concentration that requires to low 50% of ACE activity as assessed by bicinchoninic acid assay utilizing bovine serum albumin as a reference (IC_{50}) [8].

2.7.2 *In vivo* evaluation of Antihypertensive Activity

2.7.2.1 Dexamethasone induced hypertension

The Wistar rats were feed pellet meal & water, their behavior was monitored daily. On day one, every rat was weighed and their weights were recorded. They were given a positive control (dexamethasone) and a vehicle for about 14 days in a row in order for them to reach hypertensive state. The MESA (300 mg/kg) and regular amlodipine (3 mg/kg b.w, *p.o.*) medication was started on the eighth day and continued until the fourteenth day.

Study design of dexamethasone induced hypertension method is Group –I serves as Control (Normal saline) were as Group –II receives Dexamethasone (10 µg/rat *s.c.*) Group –III receives methanolic extract of *Syzygium alternifolium* (300 mg/kg, *p.o*) and Group –1V receives Amlodipine (3 mg/kg/day, *p. o.*). Venous blood pressure, heart beat and arteriolar blood pressure was measured by the tail–cuff method [9].

2.7.2.2 Fructose-induced hypertension

Rats were randomly allocated to groups at 6 weeks of age, and pulse rate and SBP were monitored every 3 days. The animals were decapitated at the conclusion of the experiment, and blood samples were taken for biochemical analysis.

The dexamethasone induced hypertension approach features a unique study design. Group –I receives normal saline as a control, while Group –II receives Fructose (10% *p.o.*) caused hypertension. The methanolic extract of *Syzygium alternifolium* (300 mg/kg, *p.o.*) is given to Group III, whereas Amlodipine (3 mg/kg/day, *p. o.*) is given to Group 1V. An automatic plasma analyzer was used to determine the levels of glucose and triglycerides in the blood. The radio-immunologists measured plasma insulin levels [10].

2.8 *In silico* analysis

i) Molecular docking

The mechanism of binding of drug with the target protein is called docking. Docking utilization to find inhibitors for specific target proteins and thus to design new stable drugs from docking results. Docking may calculate by binding energy (energy release during protein and ligand interaction). In this project, mCule software was used for docking.

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a. Structure based drug design

Initially, the protein was created by selecting any one of the chains from the PDB. Water molecules are removed from the chains. Attributes were chosen. Protein-ligand docking was performed for proteins 1EVE, 6KZP, and 3OWN using the mCule online software.

b. mCule docking results

Docking results show that some of our compounds bind well to ACE I inhibitors (PDB ID: 1EVE), Calcium channel blockers (PDB ID: 6KZP), and Renin inhibitors (PDB 3OWN)

ii) Ramachandran plot

The PROCHECK validation server generated a Ramachandran plot, which was used to assess the model's quality by having a look on the allowed & banned parts of the plot [11].

2.9 Statistical analyses

The Results were expressed as the mean ± S.E.M. The significance of the results was calculated using ANOVA and Dunnett's test and results were deliberated statistically noteworthy when significant $p<0.0001$, $p<0.001$, $p<0.01$, ns- non significant.

3. RESULTS AND DISCUSSION

Methanolic extract of *Syzygium alternifolium* bark was evaluated for its *in vitro* and *in vivo* antihypertensive activity utilizing applicable animal models. Below, the results procured from the study are given.

3.1 Percentage yield of RBME obtained by simple distillation

The MESA bark was prepared by simple distillation method. The % yield of the extract was calculated by utilizing the following formula. i.e.,

$$\% \text{ Yield of extract} = \frac{amount\ of extract\ obtained}{amount\ of\ powder\ used} * 100$$

$$= 18\ \%\ w/w$$

3.2 Preliminary Phytochemical analysis

Flavonoids, Alkaloids, glycosides, terpenoids, steroids, carbohydrates, proteins and tannins were found in the *Syzygium alternifolium*, methanolic extract according to early phytochemical analysis.

3.3 Acute toxicity studies

On albino swiss mice, a methanolic extract of *Syzygium alternifolium* was evaluated up to a level of 2000 mg/kg bd. wt. Up to 2000 mg/kg bd. wt., the animal showed no symptoms of toxicity or fatality. As a result, up to 2000 mg/kg bd. wt. of the extract was proven to be safe.

The antioxidant activity of the plant extracts was determined using a DPPH radical scavenging test and a superoxide radical scavenging assay.

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3.4 Anti-oxidant activity

3.4.1 DPPH radical scavenging activity

Table 1 show that the percent inhibition increases as the dose of plant extract is raised, however the responses are not linear for the three dose levels. Inhibition data is further processed to determine relative inhibition compared to the standard. The greater the DPPH radical scavenging activity, the higher the relative inhibition, and the benchmark utilised is ascorbic acid. At lower doses (1-5 g/mL), ascorbic acid produced inhibition, as expected. At higher dose levels (5-25 g/mL), the Methanolic extract of *Syzygium alternifolium* showed substantial inhibition, with a relative percent inhibition of 30% of the standard.

Table 1: DPPH radical scavenging activity of MESA

Test extract/standard	Dose (µg/mL)	Percentage inhibition AM±SEM (n=3)	% Relative inhibition	IC_{50} (µg/mL)
Methanolic extract of *Syzygium alternifolium*	5	28.97 ± 0.0290	34.36	12.34
	10	44.23 ± 0.0290	30.99	
	25	80.78 ± 0.3844	27.50	
Ascorbic acid (Standard)	1	16.86 ± 0.0788	100	4.08
	2.5	35.68± 0.2747	100	
	5	58.74 ±0.1362	100	

3.4.2 Superoxide radical scavenging activity

Table 2 shows the % inhibition and IC_{50} of the Methanolic extract of *Syzygium alternifolium*. Table 2 shows that the percent inhibition increases as the dose of plant extract increases, although the responses are not linear for the three dose levels. The greater the superoxide radical scavenging activity, the higher the relative inhibition. Gallic acid is used as the standard in this investigation. Gallic acid showed inhibition at lower dose levels (0.25 to 0.75 g/mL), as expected. Plant extracts also showed significant inhibition at higher dose levels (5-

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25 g/mL) and had a relatively low relative percent inhibition compared to Gallic acid. The standard and test IC_{50} values are compared. Gallic acid has a low IC_{50} value (0.64), whereas MESA has a high IC_{50} value. The relative percent inhibition decreases at increasing doses, implying that the entire dose is not used to elicit the response and that the dose response for the three dose levels is not linear.

Table 2: Superoxide radical scavenging activity of MESA

Test compound/ Standard	Dose (µg/mL)	Percent Inhibition AM±SEM (n-=3)	% Relative inhibition	IC_{50} (µg/mL)
Methanolic extract of *Syzygium alternifolium*	5	27.58±0.0464	4.47	21
	10	36.60±0.0288	4.16	
	25	58.17±0.0577	3.17	
Gallic acid (Standard)	0.25	30.80±0.0057	100	0.64
	0.5	43.89±0.0081	100	
	0.75	54.99±0.0057	100	

Following the effectiveness of free radical scavenging activities in two models, researchers attempted to investigate anti-hypertension activity in *in vitro* models.

3.5 Antihypertensive properties

Antihypertensive efficacy was assessed *in vitro* using an angiotensin converting enzyme inhibitor, as well as *in vivo* utilising dexamethasone and fructose-induced hypertension models.

3.5.1 *In vitro* ACE Inhibitory Activity

According to Table 3, the percent inhibition for *Syzygium alternifolium* extract increases with increasing dose; however, the responses for 100 and 500 mg/kg are not proportional for the two dose levels. The inhibition data was then processed for relative inhibition. The greater the

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relative inhibition, the greater the inhibitory activity of ACE. Captopril was used as the standard in this study, and it produced inhibition at lower dose levels (50 µg/mL) than MESA. At higher dose levels (100-500 µg/mL), the *Syzygium alternifolium* extract also showed significant inhibition. The methanolic extracts of *Syzygium alternifolium* showed relative percent inhibition to captopril at 30%.

Table 3: *In vitro* ACE inhibitory activity of MESA

Test compound/ Standard	**Dose (µg/mL)**	**Percent Inhibition AM±SEM (n=3)**	**% Relative inhibition**	**IC_{50} (µg/mL)**
Methanolic extract of *Syzygium alternifolium*	100	35±0.05	79.5	450
	500	52±0.21	93.9	
Captopril (Standard)	50	47.51±0.01	100	30.07

3.5.2 In vivo Antihypertensive Activity

Two *in vivo* models were used to assess antihypertensive activity: dexamethasone-induced hypertension and fructose-induced hypertension.

3.5.2.1 Effect of *Syzygium alternifolium* extract on dexamethasone induced hypertension

Following the discovery that the *Syzygium alternifolium* methanolic extract inhibited ACE, further research was conducted to assess *in vivo* antihypertensive activity. Table 4 shows that when rats were given *Syzygium alternifolium* methanolic extract (300 mg/kg) at one dose level, their systolic hypertension, diastolic hypertension, and heart beats lowered within 13 days when compared to the dexamethasone group. When compared to the MESA group, the standard amlodipine (3 mg/kg) showed lower systolic BP, diastolic BP, and beats per minute. These findings are consistent with the fundamental principle.

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Table 4: Effect of MESA on the antihypertensive parameters in dexamethasone induced hypertension in rats

Group	Dexamethasone induced hypertension, AM±SEM (n=6)			
	Blood Pressure	**Systolic Blood Pressure**	**Diastolic Blood Pressure**	**Beats Per Minute**
Control	110.0±0.705	114.9±1.67	92.48±1.70	229.9±3.794
Dexamethasone (0.2 ml *s.c*)	141.1±0.96*	126.0±1.64**	117.9±1.23*	321.8±1.265*
MESA 300 mg/kg, b.w.	114.0±1.45^{***a} ns	117.1±1.37$^{ns\ b}$	104.6±1.15^{***a} ns	267.4±1.20^{*aA}
Amlodipine 3 mg/kg, b.w.	111.92±0.830 a ns	116.88±0.86^{nsb}	95.88±0.51$^{ns\ a}$	240.1±5.46 $^{a\ ns}$

Values were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, negative control & standard. Significant values are expressed as control group (*p=0.0001, **=p<0.005, ***=p<0.01) Disease control (a=p=0.0001, b=p<0.005) & Amlodipine (A=p<0.0005), ns=non-significant.

3.5.2.2 **Effect of *Syzygium alternifolium* extract on hypertension induced by fructose**

Table 5 shows blood pressure data after treatment with *Syzygium alternifolium* extract at one treatment level (300 mg/kg, b.w.). Feeding of fructose elevated blood pressure values in rats. Following 42 days of using the *Syzygium alternifolium* extract blood pressure levels, Systolic blood pressure, Diastolic blood pressure, and heart rate significantly reduced. When compared to the standard, the test sample findings are significant.

Table 5: Effect of MESA on the antihypertensive parameters in fructose fed albino wistar rats

Group	Antihypertensive parameters AM±SEM, (n=6)			
	Blood pressure	**Systolic Blood pressure**	**Diastolic blood pressure**	**Heart rate**

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Control	103.3±3.34	105.0±2.5	72.8±1.78	221±1.31
Fructose	157.0±2.29**	144.2±3.54**	126.0±1.43**	322.5±2.20**
MESA 300 Mg/kg, b.w.	106.4±3.28a ns	115.5±1.88*** a ns	86.2±0.83**Aa	233.8±3.60*Ab
Amlodipine 3 mg/kg, b.w.	105 .0±1.34a ns	108.1±1.42a ns	75.3±2.5 a ns	225.3±2.00 a ns

Values were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, negative control & standard. Significant values are expressed as control group (*=p<0.0005, **=p=0.0001, ***=p<0.01) Disease control (a=p=0.0001) & Amlodipine (A=p<0.0005, B=P=0.05), ns=non-significant.

3.5.2.3 Effect of Syzygium alternifolium methanolic extract on fructose-induced biochemical alterations in hypertension

Table 6 shows the glucose levels after treatment with *Syzygium alternifolium* methanolic extraction at one desired concentration (300 mg/kg, b.w.). When compared to the fructose-induced high blood pressure group, glucose, triglycerides, and insulin levels decreased after treatment with *Syzygium alternifolium* extract within 42 days. When compared to the fructose group, amlodipine (3 mg/kg) showed a substantial reduction in glucose, triglycerides, and insulin levels.

Table 6: Effect of MESA on biochemical parameters in fructose induced hypertension

Groups	**AM±SEM (n=6)**		
	Glucose (mg/dL)	**Triglycerides (mg/dL)**	**Insulin (IU/mL)**
Control	86.69±0.068	135.2±0.13	2.843±0.21
Fructose	167.54±0.32*	327.7.±0.41*	4.452±0.16**
MESA 300 mg/kg, b.w.	97.83±0.26*aB	173.7±0.27*aA	4.32±0.13a ns
Amlodipine 3 mg/kg, b.w.	88.32±0.54***a	166.5±0.28*a	3.235±0.20a ns

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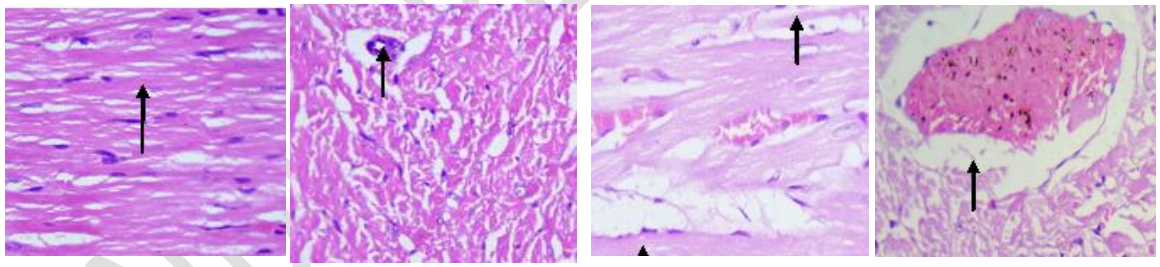
Values were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, negative control & standard. Significant values are expressed as control group (*=p=0.0001, **=p=0.001, ***=p<0.05) Disease control (a=p=0.0001) & Amlodipine (A=p=0.0001, B=p<0.005), ns=non-significant.

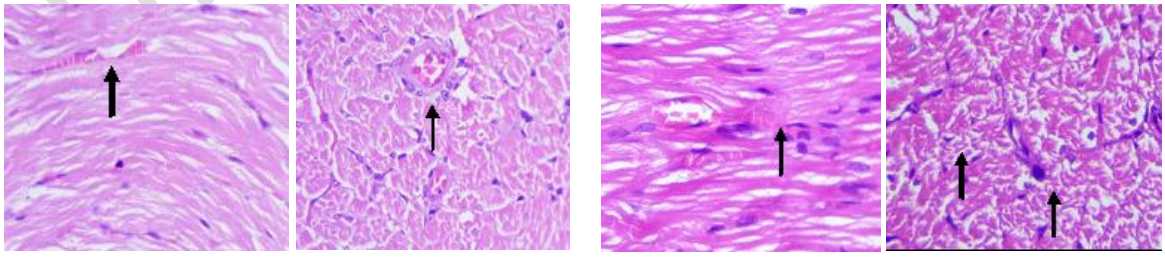
3.6 Histopathology report

3.6.1 Myocardium Histopathology Assessment

- The myofibrillar structure with striations and the integrity of the myocardial cell membrane are intact in these cardiac muscle fibres. In the myocardium of control rats, the interstitial space appears to be intact.
- • The myocardium in the induced group has a somewhat random layout and necrosis, myofibrillar structure is enlarged in focal locations, and thrombosed vascular spaces are visible.
- MESA's myocardium displays an intact arrangement of cardiac muscle fibres with a few cardiac muscle fibres displaying necrosis and increased interstitial space at focal regions. Some vascular areas appear to be clogged.
- The heart muscle fibres in amlodipine's myocardium are in good shape. The myocardial cell membrane is intact, the myofibrillar structure with striations is intact, and the interstitial gap appears to be intact in these cardiac muscle fibres.



CONTROL **INDUCED GROUP**



MESA TREATED GROUP **AMLODIPINE TREATED GROUP**

Figure 1: Histopathology of myocardium of various groups in fructose induced hypertension model

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3.6.2 Arteries Histopathology Reports

- The arterial layers seem to be intact. The tunica intima is made up of sub-endothelial connective tissue that lines the endothelium. Myocytes cells are present in the tunica media. In the control group, the tunica adventitia is a loose meshwork of connective tissue with blood vessels.
- The layers of the artery appear somewhat disturbed in the stimulated group. The tunica intima is made up of lining endothelium, with some foamy macrophages visible in the sub-endothelial connective tissue. Smooth muscle cells are present in the tunica media. The tunica adventitia is made up of hyalinized blood vessels and regions of haemorrhage.
- MESA's arterial layers seemed to be intact. The tunica intima is made up of lining endothelium, while the vascular endothelium connective tissue contains foamy macrophage clumps. Smooth muscle cells are present in the tunica media. The tunica adventitia is made up of mononuclear inflammatory cells that are dispersed throughout the tunica.
- Amlodipine's arterial layers appear to be intact. The tunica intima is made up of lining endothelium, subendothelial connective tissue, and foamy macrophages in small numbers. Smooth muscle cells are present in the tunica media. The tunica adventitia is a loose connective tissue meshwork with blood vessels.

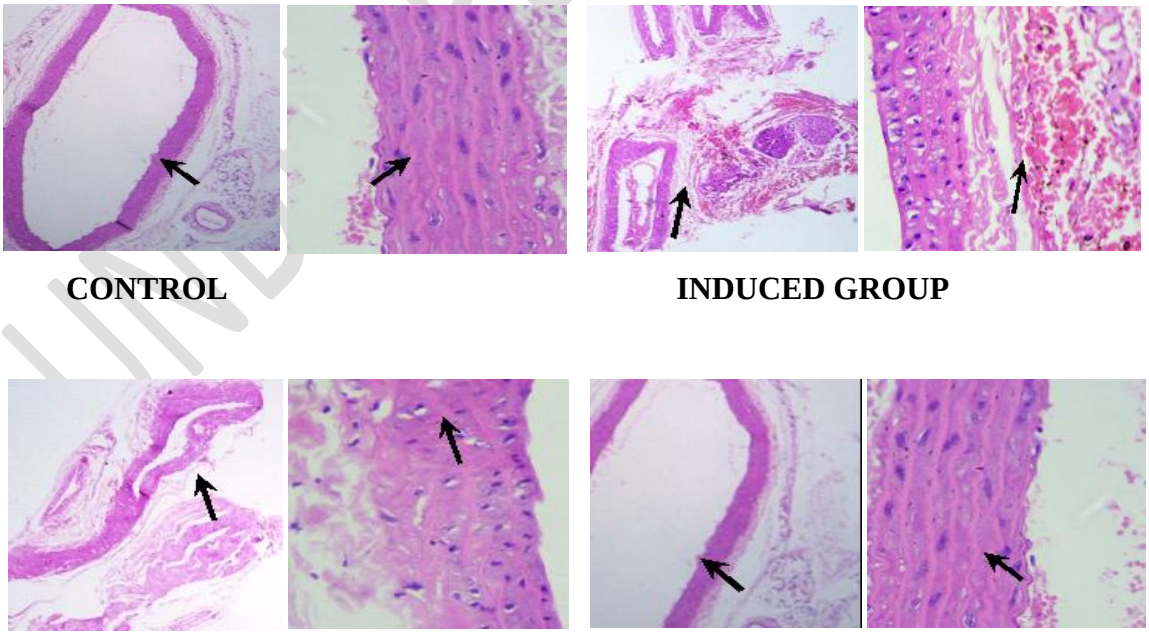

Figure 2: Histopathology of arteries of various groups in fructose induced hypertension model

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3.7 *Insilico* analysis

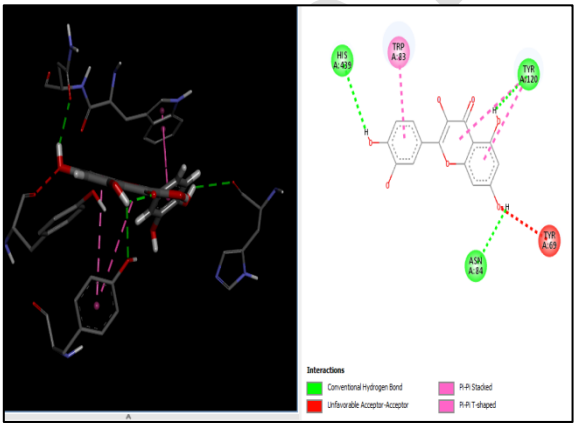
Table 7: Docking score of chemical constituents and amlodipine with protein 1EVE, 6KZP, 3OWN.

Compounds	1EVE	6KZP	3OWN
Squalene	-9.4	-7.2	-7.5
Acarbose	-8.8	-7.4	-6.6
Quercetin	-10.4	-8.1	-7.2
Kaempferol	-10.2	-7.4	-7.4
Lutidine	-5.5	-4.5	-4.8
Apigenine	-10.2	-7.7	-7.4
2,5 monomethylene-1-rhamnitol	-6.3	-5.4	-5.3
4 oxo 5 phenyl pentanoic acid	-7.3	-5.5	-6.7
Caffeic acid	-7.5	-5.8	-6.5
Gentistic acid	-6.6	-5.2	-6.1
m hydroxyl bezoic acid	-6.3	-4.9	-5.3
Amlodepin	-8.1	-7.52	-5.6

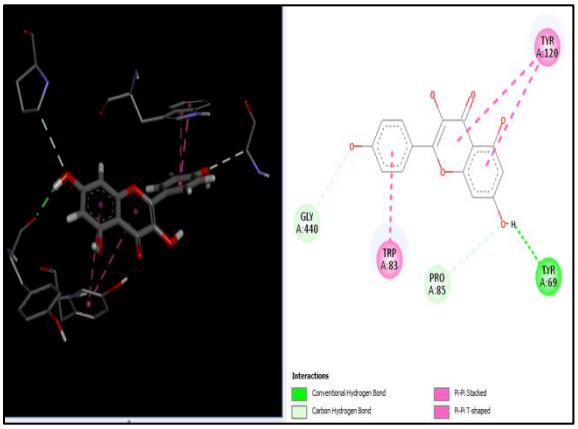
G score = glide score, Higher the negativity, the more favourable the binding.

Hydrophobic bond interactions of ligands with 1EVE, 6KZP and 3OWN protein

PDB ID: 1EVE



A) Quercetin -10.4



B) Kaempferol -10.2

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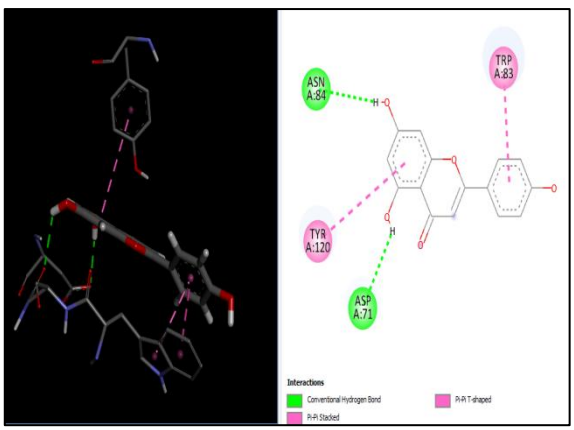
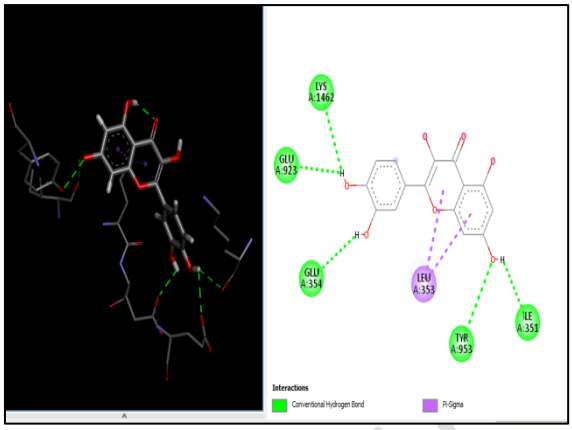
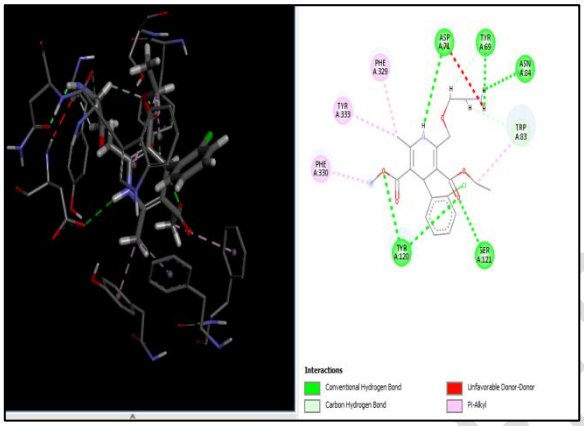


C) Apigenin -10.2

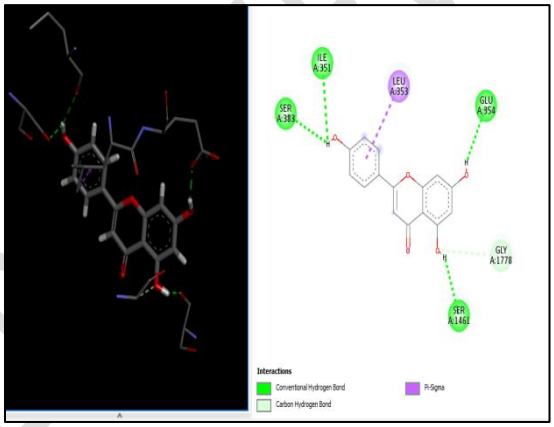


D) Amlodipine -8.1

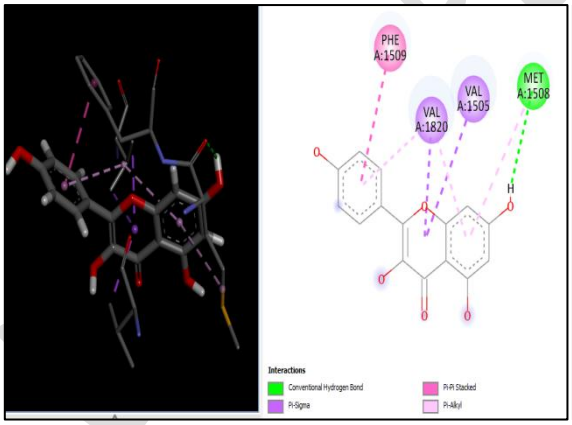
PDB ID: 6KZP



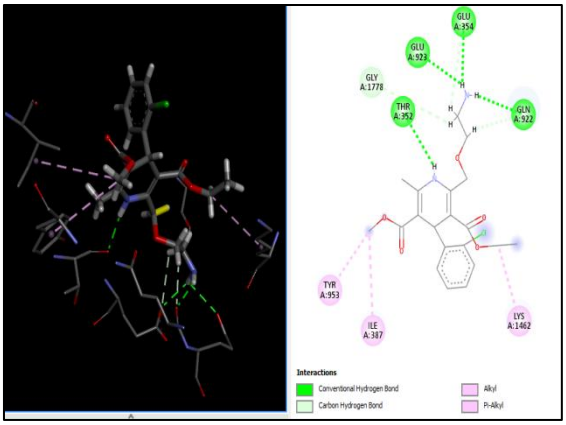
A) Quercetin -8.1



B) Apigenin -7.7

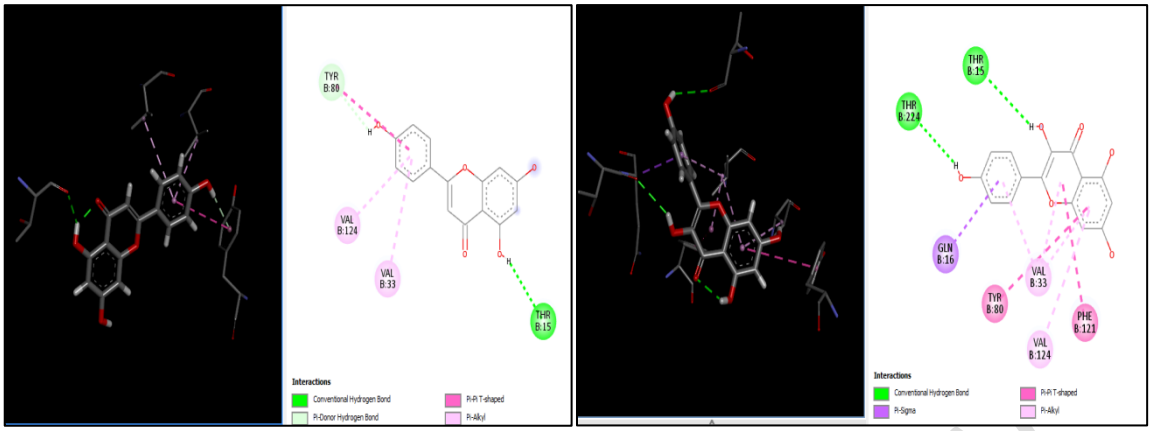


C) Kaempferol -7.4

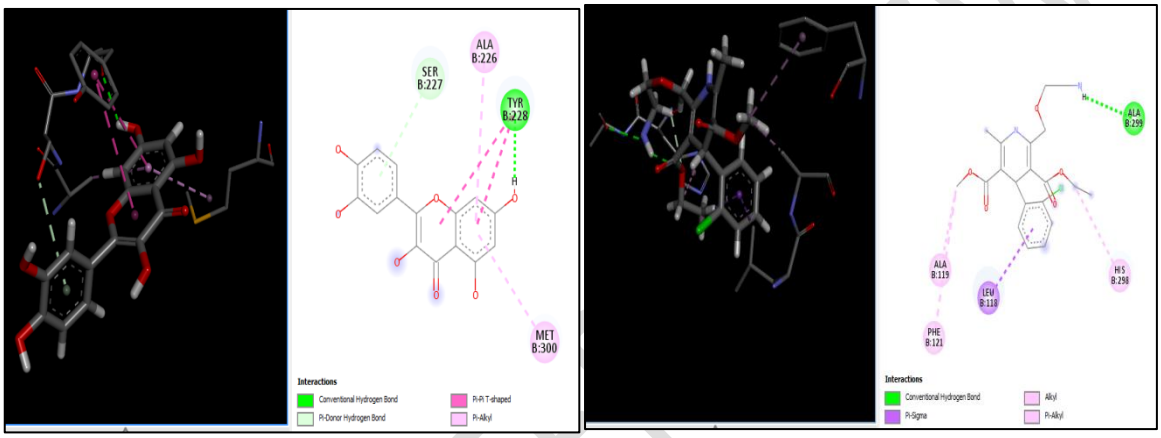


D) Amlodipine -7.52

PDB ID: 3OWN



A) Apigenin -7.4 B) Kaempferol -7.4



C) Quercetin -7.2 D) Amlodipine -5.6

Figure 3: Hydrophobic interactions of constituents and amlodipine with the 1EVE, 6KZP and 3OWN protein

ii) Analysis of the Ramachandran plot

1EVE, 6KZP, and 3OWN are proteins analysed for Ramachandran plots to determine amino acid presence in various areas of each protein, as shown in table 6 and the figure below.

Table 8: Ramachandran plot status with 1EVE, 6KZP, and 3OWN proteins

Residues	1EVE	6KZP	3OWN
Most favourable region (%)	87.3	92.1	92.1
Additional allowed regions (%)	11.8	7.4	7.7
Generously allowed regions (%)	0.7	0.4	0.2
Disallowed regions (%)	0.2	0.0	0.0

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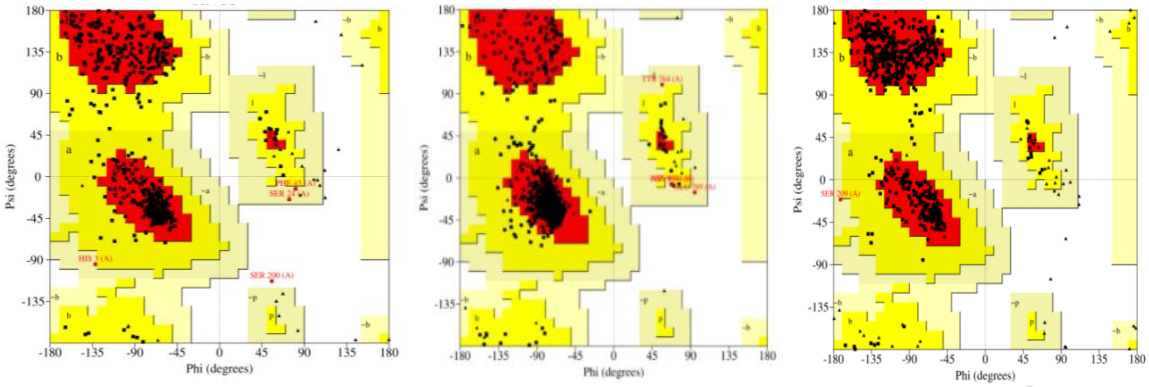

a) 1EVE b) 6KZP c) 3OWN

Figure 4: Ramachandran plot of protein 1EVE, 6KZP and 3OWN protein

4. DISCUSSION

In Dexamethasone and fructose induced hypertension models the methanolic extract of *Syzygium alternifolium bark* was tested for anti-hypertensive properties *in vitro* and *in vivo.* MESA was shown to be safe after preliminary phytochemical investigation and in acute toxicity testing.

Chronic use of the glucocorticoid dexamethasone causes hypertension, which is accompanied by increased endothelin, renin-angiotensin, sympathetic system, and hemodynamic changes [12]. Mineralocorticoid receptors have a low affinity for dexamethasone. Dexamethasone raises blood pressure in men without having any mineralocorticoid effects, as seen with the absence of hypovolemia in the urination and an increase in physical weight [13].

Fructose consumption has risen in recent decades, and it is suspected to be contributing to the expanding epidemic of metabolic diseases. Several animal experiments have shown that eating fructose promotes sodium and chloride absorption, resulting in a salt overload that raises blood pressure [14]. Hypertension has risen in relation to the increase in fructose consumption. This is in line with earlier research that has found that increasing dietary NaCl reduces renin expression and activity [15]. Finally, following 24 hours of fructose feeding, rats' urine sodium chloride excretion was found to be less than half that of the control group. Hypertension is thought to be exacerbated by increased salt absorption by the gut and decreased salt excretion by the kidney in fructose-fed mice. In fructose-fed hypertensive rats, angiotensin II (Ang II), a vasoconstrictor, is up regulated. Ang II works by connecting to the angiotensin type I and II receptors (AT1 and AT2), while the majority of its well-known effects are accomplished through interactions with AT1 [16,17].

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DPPH scavenging activity and superoxide radical scavenging activities were used to assess antioxidant activity *in vitro*. Both plants contain triterpenoids, which are responsible underlying its antioxidant properties. Antioxidant action is thought to be mediated by phenolic and flavonoids. The phenolic hydroxyl group is responsible for antioxidant activity in flavonoids, which are the most naturally occurring chemicals. These prevent the chain reaction from producing free radicals. Synergistic effects in DPPH scavenging activity and superoxide radical scavenging activity were discovered in herbal formulations, as opposed to separate components.

ACE inhibitors block an angiotensin-converting enzyme, which converts angiotensin I to angiotensin II, according to *in vitro* ACE inhibitory action. Reduced angiotensin II production improves natriuretic, decreases blood pressure, and inhibits smooth muscle and cardiac myocyte remodelling [18].

When given at a dose of 300 mg/kg, MESA reduced the systolic blood pressure, diastolic, elicited significance response with standard amlodipine. MESA decreased the Glucose, triglycerides and insulin levels in serum compared to disease group and showed significance response with control group. Compounds present in *Syzygium alternifolium* might be responsible for antihypertensive action majorly flavonoids than others That compounds are selected for docking are Quercetin, Kaempferol, Lutedine Apigenin, Acarbose and polyphenols and standard amlodepine are docked with ACE 1 inhibitor (PDB ID: 1EVE), Calcium channel blocker (PDB ID: 6KZP) and Renin inhibitor (PDB 3OWN) and Ramachandran plot analysis is done. Angiotensin converting enzyme inhibitor prevents the conversion of Ang I to Ang II so antihypertension activity is exhibited. Renin Inhibitors stops conversion of angiotensinogen to ang I and antihypertensive action is achieved. Amlodipine is a peripheral vascular dilator that acts directly upon vsmcs to reduce systemic vascular resistance, lowering blood pressure through calcium channel blocker activity. Histopathology of myocardium and arteries showed less necrosis with few vascular spasms and few macrophages compared to Disease group. Amlodipine showed antihypertensive results.

Flavonoids are a type of secondary metabolite found in plants that has a number of useful pharmacological effects. Scientists investigated the possible use of flavonoids including flavonoid-rich extracts as biological ACE inhibitors after learning about their effectiveness as biomolecules. ACE activity has been recognised as a vital factor in regulating excessive blood pressure [19]. Compounds like Quercetin, Kaempferol and apigenin known to exhibit good docking score compared to others including standard

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amlodipine. Ramachadran plot resulted the presence of amino acid in most favourable region is greater than 87%. In the present study the superposition of Quercetin, Kaempferol and apigenin and other compounds docking found with ACE 1 inhibitor (PDB ID: 1EVE), Calcium channel blocker (PDB ID: 6KZP) and Renin inhibitor (PDB 3OWN) protein have certified the precision of present docking study and Ramachandran plot that resulted anti-hypertensive activity.

5. CONCLUSION

The methanolic extract decreased the systolic vascular constriction, diastolic vasoconstriction and number of beats in minute significantly in dexamethasone induced group. The BP was also diminished (systolic, diastolic and heart rate) in rats with fructose induced hypertension. Histopathological studies revealed antihypertensive activity in response to preliminary constituents identified as Quercetin, Kaempferol, Apigenin, Acarbose and Squalene apart from other compounds as they elicited good docking score and Ramachandran plot is analysed where >87% amino acids are in favourable region. The methanolic extract of *Syzygium alternifolium* was examined for antihypertensive effects *in vitro* and in animal models, as stated in the study's objectives. The aims are achieved since the results are still extremely valuable.

ETHICAL APPROVAL

The ethical clearance for the research entitled "Antihypertensive and *In silico* Docking studies of phytoconstituents isolated from *Syzygium alternifolium* Bark" was approved by the Institutional Animal Ethics Committee of GRCP bearing Regd no. 1175/PO/Re/S/08/CPCSEA. All the animal experimentation was performed as per the guidelines of CPCSEA.

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