ORIGINAL ARTICLE

In vitro Evaluation of Selected Fruit Extracts against Cardiovascular Diseases

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Abstract

Objective: Cardiovascular diseases **(**CVDs) are the number one cause of death globally, more people die annually from CVDs than from any other cause. Much of this interest centers on the use of antioxidant vitamins and the antioxidant properties of herbal materials, although some herbal materials may also improve conventional cardiovascular risk factors or have antithrombotic effects. This study was undertaken to investigate the *in vitro* evaluation of selected fruit extracts against cardiovascular metabolic syndrome with methanolic extracts of *Schisandra (Magnolia vines), Muntingia calabura,* and *Alangium salviifolium* fruits. **Materials and Methods:** Angiotensin-converting enzyme (ACE) inhibition assay, assay of nitric oxide (NO) scavenging activity, and *in vitro* α-amylase inhibitory studies were carried out to evaluate the cardiovascular metabolic syndrome activity of methanolic extract of *Schisandra (M. vines), M. calabura,* and *A. salviifolium* fruits. **Results:** The preliminary phytochemical screening showed the presence of various phytoconstituents such as flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in the fruit extracts. The ACE inhibitory activity of fruit extracts was represented as percentage ACE inhibition. The fruits extract demonstrated ACE inhibitory activity at a concentration of 800 µg/ml, showing an inhibition >50%. Statistically significant results were observed in *in vitro* α-amylase inhibitory assay and in NO scavenging assay. **Conclusion:** The role of redox mechanisms in the control of expression and activity of rennin-angiotensin system (RAS) enzymes and angiotensin receptors may provide important insight into the function of local tissue RAS in health and disease states. The selected fruit extracts have promising role against CVDs.

Key words: *Alangium salviifolium*, cardiovascular metabolic syndrome activity, *Magnolia vines*, *Muntingia calabura*

INTRODUCTION

The cardiovascular disease (CVD) is common, morbid, and responsible for about 17.3 million deaths annually worldwide.[1] The modifiable risk factors for CVD include obesity, hypertension, hyperlipidemia, type 2 diabetes mellitus, MetS, and lifestyle risk factors such as smoking, physical inactivity, and dietary factors.[1,2] The nutritional factors have an important bearing on the cardiovascular (CV) health, either directly or through their effects on various CV risk factors including hypertension, dyslipidemia, and diabetes mellitus. The protective effects against CVD have been demonstrated for various nutraceuticals and dietary supplements,[3] and these simple lifestyle interventions open practical, potentially easy, and affordable possibilities for population-based strategies for CVD risk reduction. Epidemiological and clinical data: Epidemiological and clinical studies indicate that the risk of CVD is reduced by a diet rich in fruits, vegetables, unrefined grains, fish, and low-fat dairy products, and foods low in saturated fats and sodium are helpful. Other foods such as mono- and polyunsaturated fats, brans, nuts, plant sterols, and soy proteins have all been shown to have a favorable effect on lipid profile and blood pressure, and the overall CV health. Foods and nutrients play a key role in functioning of various

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body organs and are helpful in maintaining health and reducing the risk of various diseases.

The nutraceutical is defined as "a food component that provides potential medical or health benefits, including the prevention and treatment of disease."[4] The definition includes medicinal products made from natural ingredients. The nutraceuticals supplement the diet and also aid in the prevention and/or treatment of disease. Early research evaluated the benefits of plant-derived foods based on their Vitamin C, Vitamin E, and carotenoid content. More recent work pointed out correlation of benefits with individual compounds. However, the effects noted by testing them alone may be related to the synergistic action of the myriad of other bioactive components present in foods. In each family of bioactive compounds, there are usually various members present.

Rennin-angiotensin system plays an important role in the normal control of CV and renal function, but the system also becomes a contributing factor in the development and progression of various types of CVD, including hypertension and heart failure. The links between angiotensin (Ang) pathways and nitric oxide (NO) production and signaling effects centers on Ang-(1–7) and activation of its specific receptor Mas, a G-protein coupled receptor encoded by the Mas protooncogene. Mas receptor stimulation activates PI3K-dependent Akt phosphorylation, leading to NO production by phosphorylation of endothelial NO synthase.[5] Mas activation can also lead to NO production through neural NO synthase,[6] although the intermediate pathways are not yet defined. Ang-(1–7)/Mas receptor activation can also inhibit mitogen-activated protein kinase phosphorylation, stimulate phospholipase A2 activity and consequently release of arachidonic acid for prostanoid production, and interfere in various ways with Ang II/AT1 receptor mediate signaling pathways.[7]

Sage-leaved alangium is a small, bushy tree offering a dense canopy with a short trunk. It bears fragrant white flowers which have green buds. The petals of the flower typically curl backwards exposing the multiple stamens and a linear stigma distinctly sticking out. The fruits are spherical berry like and red and the prominent remains of the calyx can be seen distinctly with a white color. The leaves are simple, alternate, and oblong-lanceolate and stunted branches end up with sharp ends making it look like thorns.[8]

Schisandra (*Magnolia vines*) is a genus of twining shrub that generally climbs on other vegetation. Some early publications misspell the name as "Schizandra," but "*Schisandra*" is correct. Various authors have included the plants in the Illiciaceae *Schisandra* is native to Asia and North America, with a center of diversity in China. Some species are commonly grown in gardens as ornamentals. It is a hardy deciduous climber which thrives in almost any kind of soil; its preferred position is on a sheltered, shady wall. It may be propagated by cutting off half-matured shoots in August. Despite its common name, *Schisandra* is not closely related to the true magnolias.[9]

Muntingia is a genus of plants in the family *Muntingiaceae*, comprising only one species, *Muntingia calabura*. It is native to the neotropics, from Mexico South to Bolivia. *M. calabura* is a shrub or tree up to 12 m tall with spreading branches. The leaves are alternate, distichous, oblong or lanceolate, 4–15 cm long and 1–6 cm wide, with toothed margin and covered in short hairs. The flowers are small (up to 3 cm wide), solitary or in inflorescences of 2–3 flowers; with 5 lanceolate sepals, hairy; 5 obovate white petals; many stamens with yellow anthers and a smooth ovoid ovary. Fruit an edible berry, red at maturity, about 1.5 cm wide.[10]

MATERIALS AND METHODS

Plant material and preparation of extract

Schisandra (M. vines), M. calabura, and *Alangium salviifolium* fruits were obtained from the local places of Tirupati, AP. The plant was authenticated by Dr. K. Madhava Chetty M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Department of Botany, SVU, Tirupati, AP.

Fruits without rind were treated with sufficient amount of pure methanol for 1 week at room temperature with occasional shaking. The extract was filtered through a cotton plug followed by Whatman No. 1 filter paper. The filtrate was then evaporated under reduced pressure to give a dark green viscous mass and stored at 4°C until use.

Phytochemical evaluation

The methanolic extraction of *M. vines* (MEMV), methanolic extraction of *M. calabura* (MEMC), and methanolic extraction of *A. salviifolium* (MEAS) were screened for the presence of various phytoconstituents such as carbohydrates, proteins, flavonoids, polyphenolic compounds, saponins, tannins, and triterpenoids.[11]

Ang-converting enzyme inhibition assay

The Ang-converting enzyme inhibitory activity was carried out using (*N*-[3-(2-furyl) acryloyl]-Phe-Gly-Gly) (FAPGG) as the substrate.[12] The extracts and the standard drug lisinopril (1 mg/ml) were prepared by dissolving in reaction buffer (HEPES 25 mM, NaCl 293 mM, pH 8.3). The assay mixture (750 µl) consisting of 530 µl of FAPGG (3 mM in reaction buffer) and 200 µl of extract at different concentrations (100–800 µg/ml) was incubated for 3 min at 37°C. The reaction was initiated by adding 20 µl of Ang-converting enzyme (ACE) solution (0.05 U/ml) to the test reaction and the samples were incubated for 1 h at 37°C. The reaction was then stopped by adding 80 µl of 5% trifluoroacetic acid solution and samples were centrifuged at 9000 rpm for 5 min at room temperature. The enzymatic activity was calculated by quantifying the decrease in FAPGG concentration by recording the decrease

in absorbance at 345 nm using RP-18 column (50 mm × 7 mm, 3 µm pore size) with isocratic elution using acetonitrile and 1.1% trifluoroacetic acid in Milli-Q in the ratio of 75:25 v/v; it was filtered through 0.45 µL filter (Sartorius, Germany) and using an ultrasonic bath was degassed before use. The column temperature was ambient and the total running time was 10 min using a flow rate of 1.5 ml/min with retention time of 2.7 min for FAPGG, the injection volume was 20 µl, and the detection wavelength was 345 nm.

Percentage enzyme inhibition was calculated by comparing the enzymatic activity with and without inhibitor using the following formula, %ACE inhibition = (1−a) ×100

Where, "a" is the activity with inhibitor and activity without inhibitor.[13,14]

Assay of NO scavenging activity

NO scavenging activity can be estimated by the use of Griess Ilosvay reaction.[15] The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations (5 - 200µg/ml) of methanol extract of each fruit was dissolved in methanol and incubated at 300 C for 2 hours. The same reaction mixture without the extract, but the equivalent amount of methanol served as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H3P04, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 550 nm. Inhibition of nitrite formation by the plant extracts and the standard antioxidant ascorbic acid was calculated relative to the control. Inhibition data (percentage inhibition) were linearized against the concentrations of each extract and standard antioxidant. IC50 which is an inhibitory concentration of each extract required to reduce 50% of the NO formation was determined.

In vitro α-amylase inhibitory studies

The assay was carried out following the standard protocol with slight modifications.[16] Starch azure (2 mg) was suspended in a tube containing 0.2 ml of 0.5 M Tris-HCl buffer (pH 6.9) containing 0.01 M calcium chloride (substrate). The tube was boiled for 5 min and then pre-incubated at 37°C for 5 min. One milliliter of 0.1% of dimethyl sulfoxide was used to dissolve 1 mg of dried plant extracts to obtain concentrations of 10, 20, 40, 60, 80, and 100 µg/ml. Then, 0.2 ml of plant extract of a particular concentration was put in the tube containing the substrate solution. 0.1 ml of porcine pancreatic amylase in Tris-HCl buffer (2 units/ml) was added to the tube containing the plant extract and substrate solution. The process was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was then centrifuged (Eppendorf-5804 R) at 3000 rpm for 5 min at 4°C. The absorbance of resulting supernatant was measured at 595 nm using spectrophotometer (PerkinElmer Lambda 25 UV-VIS). Same procedure was followed for all other plants extracts (petroleum ether, hexane, chloroform, ethanol, and aqueous) to test the alpha-amylase inhibitory effects. The experiments were repeated thrice using the same protocol.

RESULTS

The preliminary phytochemical screening showed that the presence of various phytoconstituents such as flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in MEAS, MEMV, and MEMC results is shown in Table 1.

The IC50 value fruits of MEMV, MEMC, and MEAS were 666.26 ± 1.32 µg/ml, 668.34 ± 3.12 µg/ml, and 671.13 ± 1.72 µg/ml and that of standard, lisinopril, were 0.19 ± 0.02 ng/ml [Table 1]. The extracts inhibited ACE in a concentration-dependent manner [Table 2].

The ACE inhibitory activity of MEMV, MEMC, and MEAS was represented as percentage ACE inhibition by the extracts. The fruits of MEMV, MEMC, and MEAS demonstrated ACE inhibitory activity at a concentration of 800 µg/ml, showing an inhibition >50%. Thus, the presence of phenolic

Table 1: Phytochemical screening of MEAS, MEMV, and MEMC

S. No.	Name of the phytochemical	MEMV	MEMC	MEAS
1.	Flavonoids	+	+	+
2.	Phenolic compounds	+	+	+
3.	Triterpenoids	+	+	+
4.	Tannins	+	+	+
5.	Saponins	+	+	+
6.	Alkaloids	–	+	+
7.	Carbohydrates	+	+	+
8.	Proteins	+	+	+
9.	Amino acids	+	–	–
10.	Cardiac glycosides	–	+	–

MEMV: Methanolic extraction of *M. vines*; MEMC: Methanolic extraction of *M. calabura*; MEAS: Methanolic extraction of *A. salviifolium*

and flavonoid content in the extract would have contributed toward ACE inhibition.

The results of phytochemical screening are given in Table 3. The results of NO scavenging activity of the selected plant extracts are shown as percent of NO scavenging in Table 2. Incubation of solutions of sodium nitroprusside in phosphate buffer saline at 250°C for 2 h resulted in linear time-dependent nitrite production, which is reduced by the tested methanolic extracts of MEMV, MEMC, and MEAS. This may be due to the antioxidant principles in the extract, which compete with oxygen to react with NO, thereby inhibiting the generation of nitrite.

Positive control acarbose is a known drug for alpha-amylase inhibitor. Acarbose (at a concentrations 10–100 µg/ml) showed alpha-amylase inhibitory activity from 18.75 ± 1.17 to 58.54 ± 1.20% with an IC50 value 83.33 ± 0.75 µg/ml. MEMV, MEMC, and MEAS (at a concentrations 10–100 µg/ml) showed maximum alpha-amylase inhibitory activity from 35.79 ± 0.33 to 62.49 ± 0.34%, 16.50 ± 1.23 to 66.66 ± 0.93%, and 21.57 ± 1.46 to 60.78 ± 0.55% with an IC50 value of 37.86 ± 0.32 µg/ml, 62.99 ± 1.20 µg/ml, and 59.0 ± 0.51 µg/ml, respectively [Table 4].

DISCUSSION

Flavonoids are a group of polyphenolic compounds, which have been reported to possess ACE inhibitory activity and the activity of flavonoids and other polyphenols may be due to the

Table 2: ACE inhibition assay

Extract/standard	Concentration	% inhibition	IC50 µg/ml
MEMV (µg/ml)	100	12.11±0.38	666.26±1.32*
	200	23.90±0.35	
	400	47.10±0.47	
	800	53.15±0.53	
MEMC (µg/ml)	100	13.23±0.28	668.34±3.12*
	200	24.45±0.56	
	400	48.35±0.56	
	800	54.65±0.34	
MEAS (µg/ml)	100	14.73±0.78	671.13±1.72*
	200	27.85±0.55	
	400	49.65±0.47	
	800	57.78±0.23	
Lisinopril (ng/ml)	0.1	42.12±0.23	0.19±0.02
	0.2	51.38±0.32	
	0.4	78.92±0.45	
	0.8	82.98±0.56	

ACE: Angiotensin-converting enzyme; MEMV: Methanolic extraction of *M. vines*; MEMC: Methanolic extraction of *M. calabura*; MEAS: Methanolic extraction of *A. salviifolium*; values are expressed as mean±SEM of three parallel measurements. *P<0.01 when compared with standard

Table 3: Assay of NO scavenging activity

Concentration (µg/ml)	% of scavenging of NO			
	MEMV	MEMC	MEAS	Ascorbic acid
5	13.731±0.006	20.294±0.006	25.538±0.011	49.528±0.043
25	36.816±0.018	26.896±0.023	31.083±0.009	69.974±0.014
50	51.000±0.011	30.215±0.008	33.308±0.009	77.857±0.007
100	60.166±0.015	50.924±0.013	36.929±0.016	93.361±0.019
200	60.807±0.005	53.942±0.005	50.434±0.018	96.266±0.012
IC50	48.27	97.81	196.89	5.47

NO: Nitric oxide; MEMV: Methanolic extraction of *M. vines*; MEMC: Methanolic extraction of *M. calabura*; MEAS: Methanolic extraction of *A. salviifolium*

Table 4: *In vitro* A-amylase inhibitory studies

Analyte	Concentration (µg/ml)	% inhibition	IC 50
MEMV	100	62.49±0.34	37.86±0.32
	80	61.74±0.69	
	60	60.24±0.69	
	40	58.01±0.34	
	20	44.68±0.37	
	10	35.79±0.33	
MEMC	100	66.66±0.93	62.99±1.20
	80	66.11±0.15	
	60	47.30±1.27	
	40	37.12±1.45	
	20	27.06±3.06	
	10	16.50±1.23	
MEAS	100	60.78±0.55	59.0±0.51
	80	59.21±0.55	
	60	57.25±0.55	
	40	45.86±0.95	
	20	37.25±1.46	
	10	21.57±1.46	
Acarbose	100	58.54±1.17	83.33±0.75
	80	48.27±0.38	
	60	38.74±0.64	
	40	29.73±0.60	
	20	22.41±1.38	
	10	18.75±1.20	

MEMV: Methanolic extraction of *M. vines*; MEMC: Methanolic extraction of *M. calabura*; MEAS: Methanolic extraction of *A. salviifolium*

formation of chelate complexes with the zinc atom within the active center of zinc-dependent metallopeptidases or possibly by the formation of hydrogen bridges between the inhibitor and amino acids near at the active site. Thus, the presence of phenolic and flavonoid content in the extract would have contributed toward ACE inhibition. The ACE inhibitory activity of MEMV, MEMC, and MEAS was represented as percentage ACE inhibition by the extracts. The fruits of MEMV, MEMC, and MEAS demonstrated ACE inhibitory activity at a concentration of 800 µg/ml, showing an inhibition >50%. NO is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc., and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. NO is generated in biological tissues by specific NO synthesis (NOSs), which metabolizes arginine to citrulline with the formation of NO through a five-electron oxidative reaction. These compounds are responsible for altering the structural and functional behavior of many cellular components. Incubation of solutions of sodium nitroprusside in PBS at 25°C for 2 h resulted in linear time-dependent nitrite production, which is reduced by the tested methanolic extracts of the plants. NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants. To evaluate the antioxidant potency through NO scavenging by the test samples, the change of optical density of NO was monitored.

CONCLUSION

The preliminary phytochemical screening showed the presence of various phytoconstituents such as flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in MEAS, MEMV, and MEMC. The fruits of MEMV, MEMC, and MEAS demonstrated ACE inhibitory activity at a concentration of 800 µg/ml, showing an inhibition >50%. From *in vitro* α-amylase inhibitory studies, the fruits of MEMV, MEMC, and MEAS showed maximum alpha-amylase inhibitory activity. This study states the protective role of the methanolic extract of fruits of *Schisandra* (*M. vines*), *M. calabura*, and *Alangium salviifolium* against CVDs. Therefore, fruits in human diets might serve as possible protective agents to help us reduce oxidative damage and the risk of CVD.

REFERENCES

1. Roth GA, Huffman MD, Moran AE, Feigin V, Mensah GA, Naghavi M, *et al*. Global and regional patterns in cardiovascular mortality from 1990 to 2013. Circulation 2015;132:1667-78.
2. Breda J. F and V Consumption and Life Expectancy in Europe;2015.
3. Alissa EM, Ferns GA. Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. *J Nutr Metab 2012*;*2012*:569486.
4. Zuchi C, Ambrosio G, Lüscher TF, Landmesser U. Nutraceuticals in cardiovascular prevention: Lessons from studies on endothelial function. *Cardiovasc* Ther 2010;28:187-201.
5. Sampaio WO, dos Santos RA, Faria-Silva R, da Mata Machado LT, Schiffrin EL, Touyz RM. Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. Hypertension 2007;49:185-92.
6. Yang RF, Yin JX, Li YL, Zimmerman MC, Schultz HD. Angiotensin-(1-7) increases neuronal potassium current via a nitric oxide-dependent mechanism. Am J Physiol Cell Physiol 2011;300:C58-64.
7. Höcht C, Mayer M, Taira C. Therapeutic perspectives of angiotensin-(1-7) in the treatment of cardiovascular disease. Open Pharmacol J 2009;3:1-15.
8. Shravya S, Vinod BN, Sunil C. Pharmacological and phytochemical studies of *Alangium salvifolium* Wang. -A review. *Bull Fac Pharm Cairo Univ* 2017;55:217-22.
9. Szopa A, Ekiert R and Ekiert H. Current knowledge of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia

vine) as a medicinal plant species: A review on the bioactive components, pharmacological properties, analytical and biotechnological studies. *Phytochem Rev* 2017;16:195-218.
10. Preethi K, Premasudha P, Keerthana K. Anti-inflammatory activity of *Muntingia calabura* fruits. Pharmacogn J 2012;4:51-6.
11. Khandelwal K. Practical Pharmacognosy. Pune (Maharashtra): Pragati Books Pvt. Ltd.; 2008.
12. Holmquist B, Bünning P, Riordan JF. A continuous spectrophotometric assay for angiotensin converting enzyme. *Anal Biochem* 1979;95:540-8.
13. Ojeda D, Jiménez-Ferrer E, Zamilpa A, Herrera-Arellano A, Tortoriello J, Alvarez L. Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin-and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*. J *Ethnopharmacol* 2010;127:7-10.
14. Lahogue V, Réhel K, Taupin L, Haras D, Allaume P. A HPLC-UV method for the determination of angiotensin I-converting enzyme (ACE) inhibitory activity. Food Chem 2010;118:870-5.
15. Badami S, Moorkoth S, Rai SR, Kannan E, Bhojraj S. Antioxidant activity of *Caesalpinia sappan* heartwood. Biol Pharm Bull 2003;26:1534-7.
16. Hansawasdi C, Kawabata J, Kasai T. Alpha-amylase inhibitors from roselle (*Hibiscus sabdariffa* Linn.) tea. Biosci Biotechnol Biochem 2000;64:1041-3.

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