ORIGINAL ARTICLE

Phytochemical screening and *in vitro* antioxidant study of *Magnolia vine*, *Muntingia calabura*, and *Alangium salviifolium* fruits

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Abstract

Objective: This study was undertaken to investigate the antioxidant activity of methanolic extract of *Schisandra* (magnolia vine) (MEMV), *Muntingia calabura* (MEMC), *and Alangium salviifolium* (MEAS) *fruits*. **Materials and Methods:** Rindless fruits were subjected to treatment with pure methanol in a sufficient quantity at room temperature for a period of one week with intermittent shaking. The resultant extract then underwent double filtration, first through a cotton plug and then through Whatman filters paper No. 1. Evaporation under reduced pressure was carried out on the filtrate to get a dark green viscous mass which was stored till use at 4°C. Hydroxyl radical (OH) scavenging activity determination of reducing power, lipid peroxidation induced by carbon tetrachloride, and inhibitory test on protein oxidative modification were carried out for evaluation of the antioxidant activity of MEMV, MEMC, and MEAS fruits generated methanolic extract. **Results:** The inhibitory ratio of MEMV, MEMC, and MEAS on albumin oxidative modification was as high as 78.94 at a concentration of 1000µg/ml that showed an increasing proportionality trend with concentration. The reducing power of MEMV, MEMC, and MEAS increased with increasing concentration of MEMV, MEMC, and MEAS. **Conclusion:** All the tested concentrations of MEMV, MEMC, and MEAS showed significant ($P < 0.001$) activity than control, the MEMV, MEMC, and MEAS (at all tested doses 100 µg, 200 µg, and 300 µg) significantly ($P < 0.001$) showed scavenging activity on OHs, which were generated by the ethylenediaminetetraacetic acid/H_2O_2 system, in comparison to control. A similar increase in percent scavenging of OH radicals by MEMV, MEMC, and MEAS was observed with an increase in dose.

Key words: *Alangium salviifolium*, antioxidant activity, *Magnolia vine*, *Muntingia calabura*

INTRODUCTION

Substantial evidence implicating the involvement of free radicals in metabolic syndrome development has been published.[1] Diseases such as liver cirrhosis, diabetes, and nephrotoxicity have been reported to have free radicals effect either in their development or progression.[2] These are unavoidable by-products of redox reactions occurring in the biological systems along with certain derivatives of oxygen[3] Nitric oxide, hydroxyl radical (OH), and superoxide anions all of which are reactive oxygen species cause enzyme inactivation resulting in significant cellular components damaged by covalent binding and lipid peroxidation ultimately injuring the tissue.[3] During this process, fibrosis and synthesis of collagen are augmented. All the stress conditions have an implication of enhanced oxygen derivatives toxic in nature as a common feature. To combat this hurdle, ample mechanisms consisting generation of antioxidants and enzymes have been gradually developed in the biological systems of plants and animals.

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Sage-leaved *alangium* is a bushy tree that is small, has a thick canopy and a short trunk. Its white flowers are fragrant having green buds. The berry-like fruits are spherical, red in color. Arrangement of leaves is alternate with oblong-lanceolate shape.[4]

Schisandra (magnolia vine) is a genus of twining shrub climbing on other vegetation. *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.[5]

Muntingia belonging to Muntingiaceae family is a plant genus comprising only species, *Muntingia calabura* that is either a shrub or tree that grows up to 12 m long. Arrangement of leaves which are lanceolate or oblong shaped is distichous. The fruits are edible berries that turn red on maturation.[6]

MATERIALS AND METHODS

Analytical grade solvents and chemicals were gift sample from Ranbaxy Fine Chemicals, Mumbai, India. 1, 1-diphenyl, 2-picrylhydrazyl was obtained from Sigma Chemicals, USA. The other chemicals used were sodium nitroprusside, O-phosphoric acid, 2,2-azinobis-(3-ethylbenzoABTS), sulfanilamide, potassium superoxide, ferrous sulfate, naphthyl ethylenediamine dihydrochloride, potassium chloride (KCl), thiobarbituric acid, trichloroacetic acid, butylated hydroxytoluene (BHT), nitroblue tetrazolium, dimethyl sulfoxide, ethylenediaminetetraacetic acid (EDTA), and sodium hydroxide.

Plant Material and Preparation of Extract

Schisandra (magnolia vine), *M. calabura,* and *Alangium salviifolium fruits* were obtained from the local places of Tirupati, AP. The plant was authenticated by Dr. K. Madhava Chetty, Department of Botany, SVU, Tirupati, AP.

Rindless fruits were subjected to treatment with pure methanol in a sufficient quantity at room temperature for a period of 1 week with intermittent shaking. The resultant extract then underwent double filtration, first through a cotton plug, and then through Whatman filters paper No. 1. Evaporation under reduced pressure was carried out on the filtrate to get a dark green viscous mass which was stored till use at 4°C.

Phytochemical Evaluation

The methanolic extraction of magnolia vine (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 [Table 1].[7]

In Vitro Antioxidant Studies

Hydroxyl radical scavenging activity

Study of competition between extract for OHs generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system and deoxyribose gives an estimate of OH scavenging. TBARS is formed by an attack of the OHs attack deoxyribose. Reaction mixture containing deoxyribose (2.8 mM), $Fecl_3$ (0.1 mM), H_2O_2 (1 mm), ascorbate (0.1 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), and various concentrations (MEMV, MEMC and MEAS 100, 200, and 300 µg/ml and standard mannitol 100 µg/ml) of the drug to a final volume of 1 ml was subjected to incubation at 37°C for 1 h followed by measurement of degradation of deoxyribose at 532 nm.[8]

Determination of Reducing Power

The below-specified method was used to determine the fruit extract's reducing power. Distilled water (1 ml) containing various concentrations (125,250,175 and 500 µg/ml) of extract of MEMV, MEMC, and MEAS with phosphate-buffered (2.5 ml, 0.2 M, pH 6.6) was mixed and potassium ferricyanide ($K_3Fe(CN)_6$) (2.5 ml, 1%) followed by incubation of mixture for 20 min at 50°C. A portion (2.5 ml) of trichloroacetic acid (15%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. About 2.5 ml of distilled water and ferric chloride (0.5 ml, 0.1%) was mixed with the resultant solution's upper layer (2.5 ml). This step was followed by measurement of absorbance at 700 nm with an increase in absorbance of reaction mixture an indication of an increase in reducing power.[9]

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 extract of magnolia vine, MEMC: Methanolic extraction of *Muntingia calabura*, MEAS: Methanolic extraction of *Alangium salviifolium*

Metal Chelating Activity

Metal chelation property for ferric ion (Fe^{3+}) was estimated using thiocyanate method. Here, different ratio of the extract (1:0.25–1:10 ratio) was mixed with a fixed concentration of ferric chloride (10 µg), followed by 30 min incubation of mixture. On completion of incubation was added 1 ml of potassium thiocyanate (25%) and absorbance of a reddish-brown complex, i.e. ferric-thiocyanate complex at 460 nm was measured the results of which were compared with EDTA (1:10). 2, 2-bipyridyl method was employed for the estimation of metal chelation property for ferrous ion (Fe^{2+}). Ferrous sulfate (10 µg) at fixed concentrations was mixed with different concentrations of the extract. The mixture was incubated for 30 min. At the end of the incubation, 2 ml of 2, 2-bipyridyl (1 mM) was added and absorbance of ferrous – bipyridyl complex (pink-colored complex) was measured at 525 nm. The results were compared with EDTA.[10,11]

Carbon Tetrachloride (CCl_4)-Induced Lipid Peroxidation

Rat liver (30%w/v) homogenate in ice-cold 0.15M KCl was prepared in a homogenizer. Aliquots of 0.5ml of homogenate were taken in different small conical flasks which were incubated in constant shaker bath (150 cycles/min) at 37^{0}C in a for 45 min with 1.5 ml of potassium phosphate-buffered (pH 7.4), 2ml of 0.15M KCl, MEMV, MEMC, and MEAS at 25, 50, 100, 200, and 300 µg/ml and Vitamin – E 100 µg/ml in different flasks and finally 10µl of CCl_4 was added. In case of control, both CCl_4 and drugs were not added and in some flasks, only drug was excluded. The reaction was stopped by addition of 4 ml of 10%(w/v) trichloroethanoic acid followed by centrifugation of contents at 4000 rpm for 10 min after incubation. Thereafter, 2 ml of the resultant supernatant was relocated to a graduated tube to which was added 2 ml of 0.67%w/v of thiobarbituric acid and subjected to heating for 15 min in a boiling water bath. The tubes were cooled, bringing the mixture to pH 12–12.5 with potassium hydroxide, stabilized the color developed, and the absorbency was measured at 543 nm.[12,13]

Inhibitory Test on Protein Oxidative Modification

The test sample (MEMV, MEMC, and MEAS 100–1000 µg/ml) and Vitamin – E (100–1000 µg/ml), and reaction mixture containing albumin (10 µg/ml) and 100 µM $CuCl_2$ in 50 mM Tris-HCl buffer (pH 7.4) were mixed in a total volume of 0.3 ml. This was followed by incubation of mixture at 37°C for 2 h. Next, addition of 1.6 ml of 0.125 M phosphate-buffered (pH 8.0) containing 12.5 mM EDTA and 10.0 M urea, and 0.1 ml of 50 mm phosphate-buffered (pH 7.0) containing 10 mM DTNB to the reaction mixture was done. The resultant solution was made to stand for 5 min at room temperature. The absorbency was read at 412 nm as cysteine-SH residue.[14]

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 MEAS, MEMV, and MEMC.

In Vitro Antioxidant Studies

OH scavenging activity

The MEMV, MEMC, and MEAS (at all tested doses 100 µg, 200 µg, and 300 µg) significantly ($P < 0.001$) scavenged the OHs generated by the EDTA/H_2O_2 system, when compared with that of control. A proportionate increase in percent scavenging activity of OH radicals by MEMV, MEMC, and MEAS with dose was observed. Results were comparable standard (mannitol 100 µg), ($P < 0.001$). Table 2 depicts the results.

Determination of Reducing Power

The reducing power of MEMV, MEMC, and MEAS increased with increasing concentration of MEMV, MEMC, and MEAS. All the tested concentrations of MEMV, MEMC, and MEAS showed significant ($P < 0.001$) activity than control. Results were in approximate with standard (BHT) ($P < 0.001$). The results are shown in Table 3.

Lipid Peroxidation-Induced by CCl_4

Lipid peroxide formation from CCl_4 was significantly ($P < 0.001$) inhibited by MEMV, MEMC, and MEAS at all tested dose levels (25 µg, 50 µg, 100 µg, 200 µg, and 300 µg), when compared with that of control. The percentage inhibitions of peroxide formation increased in a dose-dependent manner. Results were in approximate with standard ones. The results are shown in Table 4.

Inhibitory Test on Protein Oxidative Modification

The inhibitory ratio of MEMV, MEMC, and MEAS on albumin oxidative modification was as high as 78.94 at a concentration of 1000 µg/ml and increased in a concentration-dependent manner. The EC50 of MEMV, MEMC, and MEAS were 416.86 ± 0.351 µg/ml. The results were comparable with the standard (Mannitol), with a percentage inhibitory ratio of 81.99% at a concentration of 1000 µg/ml. The IC_{50} of mannitol was found to be 263.35 ± 7.41 µg/ml. The results are shown in Table 5.

DISCUSSION

The MEMV, MEMC, and MEAS (at all tested doses 100 µg, 200 µg, and 300 µg) significantly ($P < 0.001$) scavenged the

Table 2: Hydroxyl radical scavenging activity MEMV, MEMC, and MEAS and mannitol

S. No.	Concentration (µg/ml)	% Inhibition of hydroxyl radical		
		MEMV	MEMC	MEAS
1	Control	-	-	-
3	MEMV, MEMC, and MEAS (200)	75.9±11.152*	75.7±12.132*	75.6±10.187*
4	MEMV, MEMC, and MEAS (400)	81.43±14.136*	81.33±15.126*	81.53±12.131*
5	Standard (Mannitol 100 µg)	81.62±12.177*	81.62±12.177*	81.62±12.177*

Statistical significant test for comparison was done by ANOVA, followed by Dunnett's "t" test. *P<0.001, when test and standard are compared against control. Values are Mean±SEM. MEMV: Methanolic extract of magnolia vine, MEMC: Methanolic extraction of *Muntingia calabura*, MEAS: Methanolic extraction of *Alangium salviifolium*

Table 3: Determination of reducing the power of MEMV, MEMC, and MEAS and BHT

S. No.	Concentration (µg/ml)	Absorbance (OD)		
		MEMV	MEMC	MEAS
1	Control	0.086 ± 0.000392	0.082 ± 0.000291	0.089 ± 0.000172
2	MEMV, MEMC, and MEAS (500)	1.0111 ± 0.00054*	1.0122 ± 0.00067*	1.0126 ± 0.00074*
3	MEMV, MEMC, and MEAS (375)	0.9578 ± 0.00074*	0.9645 ± 0.00084*	0.9843 ± 0.00114*
4	MEMV, MEMC, and MEAS (250)	0.5361 ± 0.00087*	0.5765 ± 0.000107*	0.5564 ± 0.000109*
5	MEMV, MEMC, and MEAS (125)	0.3328 ± 0.00070*	0.3246 ± 0.00024*	0.3327 ± 0.00067*
6	BHT (500)	0.6288 ± 0.00070*	0.6288 ± 0.00070*	0.6288 ± 0.00070*
7	BHT (375)	0.4935 ± 0.0037*	0.4935 ± 0.0037*	0.4935 ± 0.0037*
8	BHT (250)	0.389 ± 0.00073*	0.389 ± 0.00073*	0.389 ± 0.00073*
9	BHT (125)	0.300 ± 0.00110*	0.300 ± 0.00110*	0.300 ± 0.00110*

Statistical significant test for comparison was done by ANOVA, followed by Dunnett's "t" test. *P < 0.001, when compared against control spectrophotometric deduction of the Fe^{3+} - Fe^{2+} transformation. Values are Mean ± SEM. BHT: Butylated hydroxytoluene. MEMV: Methanolic extract of magnolia vine, MEMC: Methanolic extraction of *Muntingia calabura*, MEAS: Methanolic extraction of *Alangium salviifolium*

Table 4: Inhibition of lipid peroxidation – induction by CCl_4 system of MEMV, MEMC, and MEAS and Vitamin – E

S. No	Concentration (µg/ml)	% Inhibition		
		MEMV	MEMC	MEAS
1	Control	-		
2	MEMV, MEMC, and MEAS (25)	32.59±2.573*	31.51±2.435*	31.52±2.354*
3	MEMV, MEMC, and MEAS (50)	42.15±3.413*	43.25±3.231*	43.45±3.233*
4	MEMV, MEMC, and MEAS (100)	52.99±9.211*	52.97±9.322*	52.99±9.212*
5	MEMV, MEMC, and MEAS (200)	60.37±12.552*	60.37±11.452*	61.37±11.532*
6	MEMV, MEMC, and MEAS (300)	68.90±14.565*	68.94±14.532*	68.87±13.432*
7	Standard (Vitamin – E)	66.27±8.205*	66.27±8.205*	66.27±8.205*

Statistical significant test for comparison was done by ANOVA, followed by Dunnett's "t" test (n=6). *P<0.001, when test and standard are compared against control. Values are Mean±SEM. MEMV: Methanolic extract of magnolia vine, MEMC: Methanolic extraction of *Muntingia calabura*, MEAS: Methanolic extraction of *Alangium salviifolium*, CCl_4: Carbon tetrachloride

OHs generated by the EDTA/H_2O_2 system, when compared with that of control. The percentage scavenging of OH radicals by MEMV, MEMC, and MEAS increased in a dose-dependent manner. Results were comparable standard (Mannitol 100 µg), (P < 0.001).

The reducing power of MEMV, MEMC, and MEAS increased with increasing concentration of MEMV, MEMC, and MEAS. All the tested concentrations of MEMV, MEMC, and MEAS showed significant (P < 0.001) activity than control. Results were comparable with the standard (BHT) (P < 0.001). MEMV, MEMC, and MEAS at all tested concentrations exhibited significant (P < 0.001) chelation, when compared against control. In similar conditions, EDTA exhibited 78.64% chelation for Fe^{2+} and 85.42% for Fe^{3+}, respectively, which is significant (P < 0.001) in

Table 5: Inhibitory test on protein oxidative modification of MEMV, MEMC, and MEAS and Vitamin – E

S. No	Concentration of (µg/ml)	% inhibition (MEMV)	IC_{50} value (µg/ml)	% inhibition (MEMC)	IC_{50} value (µg/ml)	% inhibition (MEAS)	IC_{50} value (µg/ml)
1	MEMV, MEMC, and MEAS (100)	22.46±0.380	416.86±0.351	21.36±0.380	415.76±0.421	20.46±0.380	414.85±0.261
2	MEMV, MEMC, and MEAS (200)	41.34±0.237		42.32±0.242		42.35±0.2374	
3	MEMV, MEMC, and MEAS (400)	55.24±0.174		54.34±0.123		53.64±0.174	
4	MEMV, MEMC, and MEAS (600)	64.10±0.248		63.13±0.243		66.17±0.248	
5	MEMV, MEMC, and MEAS (800)	70.93±0.165		71.94±0.132		72.33±0.165	
6	MEMV, MEMC, and MEAS (1000)	78.94±0.130		77.95±0.121		79.92±0.130	
7	Standard Vitamin – E (100)	32.15±0.079	263.35±7.47	32.15±0.079	263.35±7.47	32.15±0.079	263.35±7.47
8	Vitamin – E (200)	51.68±0.242		51.68±0.242		51.68±0.242	
9	Vitamin – E (400)	63.22±0.042		63.22±0.042		63.22±0.042	
10	Vitamin – E (600)	72.18±0.052		72.18±0.052		72.18±0.052	
11	Vitamin – E (800)	80.26±0.106		80.26±0.106		80.26±0.106	
12	Vitamin – E (1000)	81.99±0.055		81.99±0.055		81.99±0.055	

MEMV: Methanolic extract of magnolia vine, MEMC: Methanolic extraction of *Muntingia calabura*, MEAS: Methanolic extraction of *Alangium salviifolium*

comparison to control. Lipid peroxide formation from CCl_4 was significantly ($P < 0.001$) inhibited by MEMV, MEMC, and MEAS at all tested dose levels (25 µg, 50 µg, 100 µg, 200 µg, and 300 µg), when compared with that of control. The percentage inhibitions of peroxide formation increased in a dose-dependent manner. Results were comparable with that of standard. The inhibitory ratio of MEMV, MEMC, and MEAS on albumin oxidative modification was as high as 78.94 at a concentration of 1000 µg/ml and increased in a concentration-dependent manner. The EC50 of MEMV, MEMC, and MEAS were found to be 416.86 ± 0.351 µg/ml. The results were comparable with the standard (mannitol), with percentage inhibitory ratio of 81.99% at a concentration of 1000 µg/ml. The IC_{50} of mannitol was found to be 263.35 ± 7.41 µg/ml.

CONCLUSION

On the basis of *in vitro* antioxidant activity, we conclude that the fruits of *Schisandra* (magnolia vine), *M. calabura,* and *A. salviifolium* contain a wide range of phytoconstituents such as alkaloids, tannins, phenolics, proteins, and saponins, which exhibit good free radical scavenging and antioxidant activity that are considered significant with respect to possessing pharmacological effectiveness.

AUTHORS' CONTRIBUTIONS

All authors contributed equally.

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