IJPSR (2021), Volume 12, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 February 2019; received in revised form, 25 August 2020; accepted, 12 December 2020; published 01 January 2021

BIOLOGICAL SCREENING AND PHYTOCHEMICAL INVESTIGATION OF *MERREMIA EMARGINATA* LEAVES

Ch. Devadasu [*1], Rajendra Y. Prasad [2], Srinivasa P. Babu [3] and S. Gananadhamu [4]

School of Pharmacy [1], Jawaharlal Nehru Technological University, Kakinada - 533003, Andhra Pradesh India.
AU College of Pharmaceutical Sciences [2], University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam - 530003, Andhra Pradesh, India.
Vignan Pharmacy College [3], Vadlamudi, Guntur - 522213, Andhra Pradesh, India.
Department of Pharmaceutical Analysis [4], NIPER, Hyderabad - 500037, Telangana, India.

Keywords:

Antitubercular, Antioxidant, Antifungal, Cytotoxic, 8-prenylnaringenin

Correspondence to Author:
Devadasu Ch

Ph. D Research Scholar,
School of Pharmacy, Jawaharlal Nehru Technological University, Kakinada - 533003, Andhra Pradesh India.

E-mail: devdaspharma@gmail.com

ABSTRACT: The dried leaves of *Merremia emarginata Burm*. F were screened for antitubercular, cytotoxic, and anti-inflammatory activities. The antimycobacterial activity of compounds was assessed against *Mycobacterium tuberculosis* using microplate Alamar Blue assay (MABA), and the cytotoxic activity was studied on Hela (Human cervical carcinoma), MCF-7 (Human breast carcinoma) cell lines. A prenylflavonoid, 8-prenylnaringenin was isolated on silica thin layer chromatography, and the radical scavenging efficiency of the isolated compound was evaluated by DPPH radical assay. The results demonstrated that the isolated compound was found to be greater than that of BHT from lower (0.25 mM) to moderate (2 mM) concentrations and comparable to BHT at higher concentrations, up to 4 mM. The flavonoid was also found to exhibit good antifungal activity when tested against *Candida albicans* (ATCC 2091) and *Aspergillus niger* (ATCC 16404).

INTRODUCTION: *Merremia emarginata* Burm. F is a perennial, much-branched herb (creeper) [1]. It is commonly known as *Ipomoea reniformis* belongs to Convolvulaceae family. *Merremia emarginata* is a procumbent herb and possess yellow color flowers. It mainly grows in the rainy season and winter season and widely distributed all over India, especially in damp places in upper Gangetic plain, Gujarat, Bihar, West Bengal, Western- Ghats, ascending up to 900m in the hills, Goa, Karnataka in India, Ceylon and Tropical Africa.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(1).507-14

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).507-14

FIG. 1: ***MERREMIA EMARGINATA***

Thorough literature reveals *that Merremia emarginata* has proven biological activities, including antibacterial [2], antioxidant and anti-arthritis activity [3], found to contain endogenous contents of phytohormones [4], studied for toxicity

on *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) and non-target mosquito predators [5], and nephroprotective Activity [6], The plant species were found to exhibit antibacterial activity [7], antidiabetic effect [8], and anticancer activity [9], some of the works were aimed to isolate diacetyltetritol [10], and phytochemical analysis by GC-MS [11], *in-vitro* antioxidant and antimicrobial activities [12-13], HPLC-ESI-MS technique for analysis of flavonoids were also tested for the species [14].

Although several biological works have been carried out on the plant, there is no specific procedure for the isolation of phenolic constituents is observed, and moreover, the extracts prepared from several works may be provided with many kinds of plant constituents, which might have given the non-specific biological responses. The main objective of the research work is to prepare an extract that consists of biologically important plant phenolics and evaluate its antibacterial, antifungal and antioxidant potential of dried leaves of *Merremia emarginata.* The main aim of this project work is to evaluate the antibacterial, Antioxidant and Anti-fungal activity from various extracts of the dried leaves of *Merremia emarginata* and to isolate the phytoconstituents by using different chromatographic methods such as column chromatography, thin-layer chromatography and characterize them by spectroscopic methods like Mass spectrometry and Nuclear magnetic resonance spectroscopy**.**

MATERIALS AND METHODS: The leaves of *Merremia emarginata* were collected in November 2015 from Munnangi, Guntur, Andhra Pradesh, India. The plant was authenticated by Dr. Satyanarayana Raju, Department of botany and microbiology, Acharya Nagarjuna University, Guntur. All the chemicals and reagents used were of analytical grade and purchased from Merck, Mumbai, India, and from S.D-fine chem., Mumbai, India. Silica powder for TLC and column was purchased from Merck Pvt., Ltd., Chem., Mumbai. Dimethyl sulfoxide, Alamar blue reagent purchased from Gaylord chemical, ^{1}H NMR and IR spectra's were recorded on Bruker avance, and Mass spectra were recorded on Elegant LC-1100 series instrument. Gram-negative *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 25619), Gram-positive *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), fungal organism are *Candida albicans* (ATCC 2091), *Aspergillus niger* (ATCC 16404), *Mycobacterium tuberculosis* (ATCC 27294). All the stock cultures were obtained from Microlab, Maratha Mandal' Singh Institute of Dental Sciences and Research Centre, Belgaum, India. MDA-MB and DU-145 cell line was obtained from the national center for cell science, Pune, India. Gentamycin I.P. (98.75%), Ascorbic acid I.P. (99.5%), and Fluconazole I.P. (98.5%) were gifted from Hetero drugs Limited Hyderabad. DPPH, BHT, was purchased from Sigma-Aldrich, St. Louis, USA. Ethanol (Analytical Reagent) was procured from M/s SDFCL (Mumbai, India).

Extraction of Phytoconstituents: The phyto-constituents present in the leaves of *Merremia emarginata* were extracted by cold maceration process. 50 grams powder of dried leaves of *Merremia emarginata* was taken into Iodine flask. The phytoconstituents were extracted by adding 2000 ml of ethanol. The powder was extracted by keeping the flasks on orbital shaker for 24 h. To the ethanolic extract, 200ml of 0.1M HCl was added. The extracts were filtered through Whatman filter paper and collected. The process was repeated twice. About 25ml of the filtrate was transferred into a 125ml separating funnel and extracted with 3×10 ml portions of ethyl acetate. The ethyl acetate portion evaporated to dryness using a rotary evaporator and preserved in desiccators for further study.

Anti-tubercular Activity: The antimycobacterial activity of compounds was assessed against *Mycobacterium tuberculosis* using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent, and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µl of sterile de-ionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth, and serial dilutions of compounds were made directly on the plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After

this time, 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. The blue color in the well was interpreted as no bacterial growth, and the pink color was scored as growth. The MIC was defined as the lowest drug concentration which prevented the color change from blue to pink

Cytotoxic Activity by MTT Assay Method: 10 mg in 10 ml of Hank's balanced solution was prepared. The cell line was maintained in 96 wells microtiter plate, which contained MEM media supplemented with 10% heat-inactivated fetal calf serum (FCS), 5% of a mixture of Gentamycin, Penicillin (100 Units/ ml), and Streptomycin (100µg/ml) in presence of 5% CO_2 at 37 ºC for 3-4 days. After 3-4 days the supernatant was removed and replaced MEM media with Hank's balanced solution supplemented with Gentamycin, Penicillin and Streptomycin and incubated overnight. *In-vitro* growth inhibition effect of test compound was assessed by colorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. The supernatant was removed from the plate and fresh Hank's balanced salt solution was added and treated with different concentrations of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. Incubated at 37 ºC for 24 h in a humidified atmosphere of 5% CO_2, the medium was replaced with MTT solution (100 µl, 1mg per ml in sterile Hank's balanced solution) and incubated for 4 h. The supernatant was carefully aspirated, the precipitated crystals of "Formazan blue' were dissolved by adding DMSO (200 µl), and optical density was measured at a wavelength of 570 nm. The result represented the mean of three readings and was reported. The concentration at which the OD of treated cells was reduced by 50% with respect to the untreated control.

Anti-inflammatory Activity: Human red blood cell membrane stabilization (HRBC) method was used for the estimation of anti-inflammatory activity *in-vitro*. Blood was collected from healthy volunteers and was mixed with an equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3000 rpm, and the packed cells were separated. The packed cells were washed with isosaline solution, and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory properties. Different concentrations of extract (100, 200, 300 µg/ml), reference sample, and control were separately mixed with 1mL of phosphate buffer, 2 ml of hypo saline, and 0.5 ml of HRBC suspension. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted, and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in control as 100%.

Percentage protection= 100 - (OD sample / OD control) × 100

Antioxidant Assay: [15]

0.1 mM DPPH Solution: 3.94 mg of DPPH was dissolved in 100 ml of ethanol and kept it under dark for the generation of DPPH radical.

2 mM BHT Solution: 11 mg of BHT was transferred into 25ml of the standard flask, and ethanol was added up to the mark.

2 mM Sample Solution: 12.80 mg of Bakuchiol was transferred into 25ml of standard flask, and ethanol was added up to the mark.

Method: Antioxidant assay was determined by its radical scavenging ability using the stable DPPH radical. Briefly, 200 µL of methanolic solution of the test sample (1 mM) was added to 2 mL of methanolic solution of DPPH radical (0.1 mM) and total volume was made up to 3 mL with methanol having 2 mM concentration. Similarly, different concentrations of the test sample were prepared. The resulting solutions were then left to stand at 37 °C for 40 min prior to being spectrophotometrically detected at 517 nm. BHT was used as positive controls. The free radical scavenging activity (FRSA in %) of the tested samples was evaluated by comparison with a control (2 mL of DPPH radical solution and 1 mL of methanol). Each sample was measured in triplicate and averaged. The FRSA was calculated using the formula:

$$FRSA\ \% = [(A_c - A_s)/A_c] \times 100$$

Where "A_c" is the absorbance of the control and ''A_s'' is the absorbance of the tested sample after 40 min.

Antifungal Activity: To determine the susceptibility patterns of fungi against compounds of the fractions, overnight grown cultures were inoculated on the surface of solidified potato dextrose agar medium. The wells were prepared on the inoculated plates equidistantly. Each well is filled with 600 µg and 800 µg of a crude extract with the help of a micropipette. After proper diffusion of extract into the media, the plates were incubated for 72 h at 37 ºC. Zones of inhibition were measured with a ruler.

Chromatographic Isolation and Characterization: Column chromatography was carried out on a Silica column (30 cm × 2 cm glass column) packed with silica gel (Merck, Darmstadt, Germany) from the commercially available manufacturers. An aliquot of the solution of ethyl acetate extract was adsorbed on silica gel, and the solvent was evaporated after thorough triturating in a mortar and pestle. The column was packed by the wet packing method by placing about 10 g of sample onto the top of the column. The column was developed with pure ethanol: ethyl acetate (50:50) as an eluent. The column fractions, about 100ml each, were collected into fraction collectors (Iodine flasks) and labeled properly. The fractions were monitored on analytical TLC to check the number of components present in each fraction. Analytical TLC was carried out on preparative TLC plates (5 × 5 cm with 0.2mm thickness, silica gel GF_{254}, Merck, Darmstadt, Germany) cut from the commercially available sheets. An aliquot of the solution of each fraction was spotted onto the silica gel plate and allowed to dry for a few minutes. Afterward, the chromatophore was developed with ethylacetate: propanol: water (6.5:2.4:1.1 v/v**)** as a mobile phase in a previously saturated glass chamber with eluting solvents for some time at room temperature. The developed plate was dried under normal air, and the spots were examined under UV cabinet containing the radiation sources of 254 nm (short wavelength) and 365 nm (long wavelength) and iodine chamber. The UV spectrum of the purified compound was recorded from200 to 800 nm on an *ELICO* double beam spectrophotometer UV-visible spectrophotometer. ESI mass spectra were acquired from isolated compounds and characterized. Proton nuclear magnetic resonance spectra were acquired using 400 MH_Z NMR spectrometer employing TMS as an internal standard, and deuterated methanol was used as a solvent.

RESULTS AND DISCUSSION:

Extraction and Screening for Activities: Extraction of phytoconstituents from dried leaves of *Merremia emarginata* was achieved by cold maceration. Physicochemical studies Preliminary phytochemical investigation was done in order to know the main constituents present in the extract, and it was found that the extract shows positive results for alkaloids, steroids, glycosides, and flavonoids, and plant phenols.

Antitubercular Activity: All extracts exhibited good anti-tubercular activity may be due to presence of alkaloids, steroids, and glycosides, which are present in *Merremia emarginata.* The results of Antitubercular activity present in **Table 1** which indicates at the concentration of 1.6 µg/ml, aqueous extract change color from blue to pink that indicates the inhibition growth of *Mycobacterium tuberculosis*. At 0.8 µg/ml, chloroform and ethanol extract shows the minimum inhibition of microbial growth.

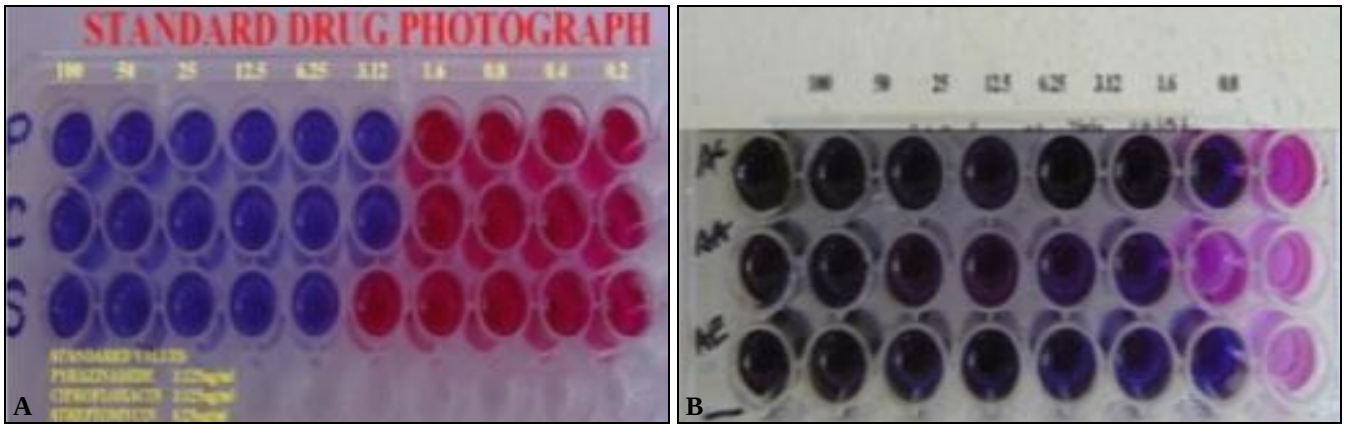

FIG. 2: ANTIMYCOBACTERIAL ACTIVITY (A) TESTED WITH ISONIAZID (B) TESTED WITH 8-PRENYLNARINGENIN

TABLE 1: RESULTS SHOWING THE ANTI-TUBERCULAR ACTIVITY OF DIFFERENT EXTRACTS OF *MERREMIA EMARGINATA*

Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
AC	S	S	S	S	S	S	S	R
AE	S	S	S	S	S	S	R	R
AA	S	S	S	S	S	S	S	R

AC- Chloroform extract, AE- Ethanolic extract, AA- Aqueous extract, R- Resistance, S- Sensitive

Anticancer Activity: For anticancer activity we preferred two cell lines; they are MDA-MB, DU-145 cell lines. The different solvent fractions of leaves of *Merremia emarginata* were subjected for MTT cell proliferation assay, and results are presented in **Tables 7** and **8**. All extract fractions of *M. emarginata* was show less anti-cancer activity on two different cell lines, it may be due to the low concentration of active constituents Caffeic acid. Purushoth prabhu *et al*., [3] reported that *Merremia emarginata* has anticancer activity by MTT assay. They prepared ethanol, chloroform, n-hexane, ethyl acetate extracts by the soxhlet extraction process. They preferred Hela (Human cervical carcinoma), MCF-7 (Human breast carcinoma) cell lines and concluded the result was ethanol and ethyl acetate extracts have potent activity in which ethyl acetate shows highly potent anticancer activity. Ethyl acetate fraction shows 51.57 µg/ml and 39.6 µg/ml IC_{50} value against Hela and MCF cells.

As per my results, anticancer activity showed less activity due to a low concentration of active constituents. Further investigation is to be carried out.

TABLE 2: RESULTS SHOWING THE CYTOTOXIC ACTIVITY OF DIFFERENT EXTRACTS OF *MERREMIA EMARGINATA* ON MDA-MB CELL LINE

Sample	Concentration (µg/ml)	Absorbance (nm)	Results as observed	IC_{50} (µg)
AE	10	1.062	No lysis	
	20	1.658	No lysis	
	25	1.640	No lysis	
	30	1.495	No lysis	
	50	1.335	No lysis	
AC	10	1.870	No lysis	
	20	2.524	No lysis	
	25	2.904	No lysis	
	30	3.228	No lysis	
	50	2.805	No lysis	
	10	1.126	No lysis	50µg
	20	1.286	No lysis	
AA	25	1.297	No lysis	
	30	1.566	No lysis	
	50	1.363	No lysis	
Control		1.127		

AC- Chloroform extract, AE- Ethanolic extract, AA- Aqueous extract, R- Resistance, S- Sensitive

TABLE 3: RESULTS SHOWING THE CYTOTOXIC ACTIVITY OF DIFFERENT EXTRACTS OF *MERREMIA EMARGINATA* ON DU-145 CELL LINE

Sample	Concentration (µg/ml)	Absorbance (nm)	Results as observed	IC_{50} (µg)
AE	10	1.051	No lysis	
	20	1.618	No lysis	
	25	1.624	No lysis	
	30	1.491	No lysis	
	50	1.335	No lysis	
AC	10	1.820	No lysis	
	20	2.142	No lysis	
	25	2.915	No lysis	
	30	3.165	No lysis	
	50	2.501	No lysis	
	10	1.111	No lysis	50µg
	20	1.278	No lysis	
AA	25	1.296	No lysis	
	30	1.566	No lysis	
	50	1.356	No lysis	
Control		1.127		

AC- Chloroform extract, AE- Ethanolic extract, AA- Aqueous extract, R- Resistance, S- Sensitive

Anti-inflammatory Activity: HRBC method was performed on chloroform extract, ethanolic and aqueous extracts of *Merremia emarginata.* The anti-inflammatory results are shown in **Table 9**. Chloroform extract at a concentration of 200 µg/ml was showed 52.5% protection of HRBC in a hypotonic solution. All the results were compared with standard diclofenac sodium, which showed 62% protection.

TABLE 4: RESULTS FOR ANTI-INFLAMMATORY ACTIVITY OF DIFFERENT EXTRACTS OF LEAVES OF *MERREMIA EMARGINATA*

Treatment	Concentration (µg/ml)	Absorbance at 560nm	% Inhibition
Control		0.080	
Chloroform extract	100	0.040	50
	200	0.038	52.5
	300	0.029	36.25
Ethanolic extract	100	0.056	30
	200	0.050	37.5
	300	0.049	38.75
Aqueous extract	100	0.058	27.5
	200	0.056	30
	300	0.040	50

Chloroform extract showed significant anti-inflammatory activity in a concentration-dependent manner. Ethanolic extract at a concentration 300

µg/ml was showed 38.75% protection of HRBC in a hypotonic solution. Aqueous extract at a concentration 300 µg/ml was showed 50% protection of HRBC in a hypotonic solution. Further studies are to be performed to know which particular active constituent is responsible for the activity.

Isolation and Characterization of 8-Prenylnaringenin:

NMR Spectra: Compound 1 is an off white amorphous powder; ^{1}H NMR data shows the presence of δ 3.7, presence of hydroxyl groups, δ 3.8, and presence of methyl groups adjacent to double bond δ 7.2-8.4, and presence of aromatic hydrogens. ^{1}H NMR spectrum of compound 2 is characterized by the δ 3.4, presence of hydroxyl groups, and δ 7.4-8.3, presence of aromatic hydrogens.

Mass Spectral Data: The total ion chromatogram and computer- reconstructed selected ion chromatograms for the negative ion electrospray mass analysis of compound 1 is shown in **Fig. 3**. Negative ion electrospray was used instead of positive ion mode because it provided enhanced signal-to-noise as well as fragmentation pathways that provided more informative product ion spectra for metabolite structure elucidation. Product ions in the tandem mass spectra are labeled according to the nomenclature of Ma *et al*., (1997) and Fabre *et al*., (2001). The major fragmentation pathway for the substrate 8-PN was found to obey Retro Diels-Alder (RDA) reaction to produce ions at *m/z* 219 and 119, respectively. This pathway was very useful for the determination of whether metabolic transformation occurred on the ring A or ring B of the flavanone system.

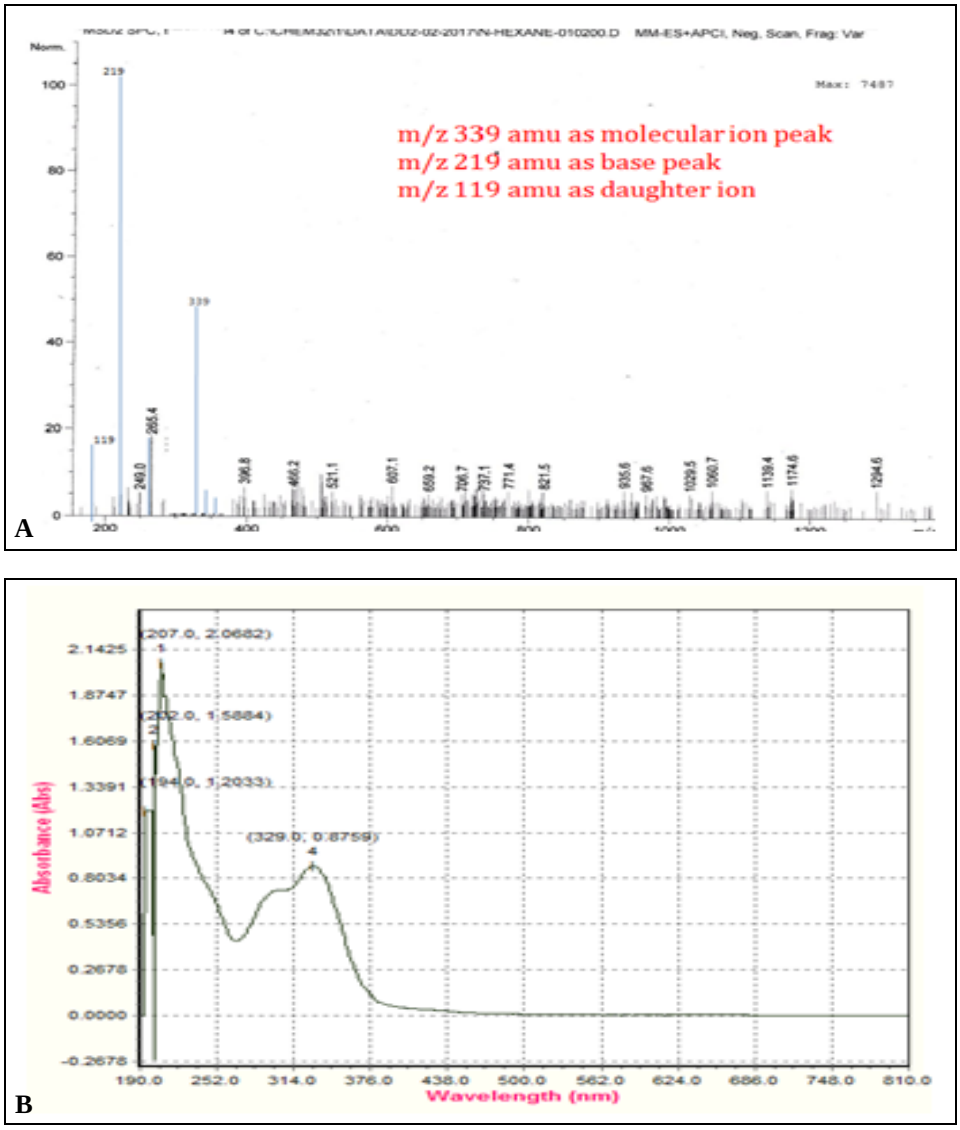

FIG. 3: SPECTRAL PROPERTIES OF ISOLATED COMPOUND-(A) MASS SPECTRUM (B) UV-VISIBLE SPECTRUM

The ion of *m*/*z* 219 fragmented further by the elimination of CO_2 to form the ion of *m*/*z* 175. Based on this loss of CO_2, Fabre *et al*., (2001) proposed that the structure of the RDA fragment ion is a lactone instead of the acyclic ketene structure that has been proposed for positive ion RDA fragment ions (Ma *et al*., 1997). Other minor fragmentation pathways were similar to those described for naringenin (Fabre *et al*., 2001) and included cleavage of the B ring (*m*/*z* 245) as well as the loss of ketene and CO_2 from the precursor ion. It should be noted that these assignments of fragment ion compositions are supported by exact mass measurements. Based on the above spectral studies, the isolated compound summarily identified as 8-Prenyl-naringenin with molecular formula $C_{20}H_{20}O_5$, and the molecular weight of the compound is found as 340 amu. UV spectrum of the compound shows its absorption maximum at about 299 nm.

TABLE 5: SPECTRAL CHARACTERIZATION OF ISOLATED COMPOUND

Spectral properties	
Physical State	Light brown-yellow colored solid
^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm)	*δ* 5.42 (1H, *dd*, *J*=12.4, 2.7 Hz, H-2), 3.19 (1H, *dd*, *J*=17.1 Hz, 12.5 Hz, H-3ax), 2.72 (1H, *dd*, *J*=17.3 Hz, 3.2 Hz, H-3eq), 5.97 (1H, *s*, H-6), 7.30 (2H, *d*, *J*=8.3 Hz, H-2' & H-6'), 6.79 (2H, *d*, *J*=8.2 Hz, H-3' and H-5'), 3.08 (2H, *d*, *J*=7.1 Hz, H-1''), 5.08 (1H, t, *J*= 7.1 Hz, H-2''), 1.59 (3H, *s*, H-4''), 1.54 (3H, *s*, H-5''), 9.50 (*s*, 4-OH), 10.70 (*br s*, 7-OH), 12.08 (*s*, 5-OH)
^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm)	δ78.2 (C-2), 41.9 (C-3), 25196.6 (C-4), 161.1 (C-5), 95.2 (C-6), 164.3 (C-7), 106.9 (C-8), 159.7 (C-9), 101.7 (C-10), 129.2 (C-1'), 128.2 (C-2'), 115.1 (C-3'), 157.2 (C-4'), 115.1 (C-5'), 21.2 (C-1''),122.7 (C-2''), 130.1 (C-3''), 17.5 (C-4''), 25.5 (C-5'')
FT-IR (neat, cm^{-1})	3376, 3081, 3024, 2966, 1609, 1512, 1235
ESI-MS (m/z)	339 $[M]^+$
UV (ethanol, nm)	329

Antifungal Activity for 8-PN: For antifungal activity we consider the organisms are *Candida albicans* (ATCC 2091), *Aspergillus niger* (ATCC 16404). The results of antifungal activity are given in **Table 5**. All the extracts exhibited anti-fungal activity against *Candida albicans and Aspergillus niger* at 75 µg/ml concentration. Among the ethanolic and chloroform extracts were found to be more active with inhibition zone diameter of 25 mm. The standard Fluconazole value is 24mm and 26mm.

TABLE 6: ANTIFUNGAL ACTIVITY OF ISOLATED COMPOUND

Extracts	Concentrations µg/ml	Zone of inhibition (mm)	
		C. albicans	*A. niger*
8-PN	75	25	20
	50	14	13
	25	10	12
	10	R	R
	5	R	R
Fluconazole	20	24	26

C. albicans (ATCC 2091), *A. niger* (ATCC 16404)

Antioxidant Activity of Isolated Compound: The radical scavenging efficiency of the isolated compound was evaluated by DPPH radical assay. DPPH free radical scavenging assay (FRSA) was based on the reduction of alcoholic DPPH solution (dark blue in color) in the presence of a hydrogen donating antioxidant converted to the non-radical form of yellow-colored diphenyl-picrylhydrazine. The advantage of this assay is that the DPPH radical is commercially available and need not be generated before the assay, like in other assays. The commercially available synthetic antioxidant BHT was used as control during the assay, and the results, as means of three independent assays, are depicted in **Table 7**. The assay was conducted at different concentrations (0.25, 0.5, 1, 1.5, 2, 3, and 4 mM) of test compounds (BHT/Bakuchiol) in a homogeneous polar medium. From the table, it was observed that 8 PN exhibited superior free radical scavenging activity (FRSA) in the range of 50.33-64.35% in the concentration range from 0.25 to 2 mM, which is far more superior to that of BHT (46.96-62.23%). At higher concentrations, *i.e.*, at 3 and 4 mM, it was observed that the compound was comparable to that of BHT.

TABLE 7: ANTIOXIDANT ACTIVITY DETERMINED BY DPPH RADICAL SCAVENGING

Compound	Concentration (mM)[a]						
	0.25	0.5	1	1.5	2	3	4
BHT	45.59±0.09	48.65±0.03	57.87±0.14	59.18±0.13	59.23±0.16	71.66±0.25	77.53±0.37
8-prenyl naringenin	49.33±0.03	55.88±0.10	57.69±0.05	60.16±0.11	64.35±0.18	68.10±0.09	74.19±0.10

[a] Values were the means of three replicates ± SD

CONCLUSION: Antioxidant assay of isolated 8-prenylnaringenin from *Merremia emarginata* leaves was evaluated by using DPPH scavenging assay. These results clearly evidence that the isolated compound was found to superior to that of BHT from lower (0.25 mM) to moderate (2 mM) concentrations and comparable to BHT at higher concentration, up to 4mM.

ACKNOWLEDGEMENT: We are thankful to the chairman, Vignan Group of Institutions, for providing research facilities throughout the research project.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

REFERENCES:

1. Prabhu TP, Sudhev S, Atlee WC and Balasubramanian S: Pharmacognostic studies on whole Plant of *Merremia emarginata* Burm. F (Convolvulaceae). International J of Pharm and Phytochemical Research 2014; 6(4): 1002-06.
2. Devadasu C, Bash SA and Srinivasa BP: Biological evaluation of *Merremia emarginata* for antibacterial, antifungal and antioxidant activities. World J of Pharmacy and Pharmaceutical Sciences 2017; 6(7): 1509-25.
3. Prabhu TP, Shine S and Venkataraman ND: *In-vitro* antioxidant and antiarthritis activity of extracts and fractions of *Merremia emarginata*. Der Pharmacia Lettre 2014; 6(6): 227-31.
4. Patel D and Thaker VS: Estimation of endogenous contents of phytohormones during internode development in *M. emarginata*. Biologia Plantarum 2007; 51(1): 75-79.
5. Azarudeen M, Thameem RMS, Govindarajan M, Mashael AlShebly M, Vijayan P and Benelli G: Size-controlled biofabrication of silver nanoparticles using *the Merremia emarginata* leaf extract: toxicity on *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) and non-target mosquito predators. Journal of Asia-Pacific Entomology 2017; 20(2): 359-66.
6. Rameshkumar A, Sivasudha T, Jeyadevi R, Sangeetha B, Ananth DA and Kathiravan A: *In-vitro* antioxidant and antimicrobial activities of *Merremia emarginata* using thio glycolic acid-capped cadmium telluride quantum dots. Colloids and Surfaces B: Biointerfaces 2013; 101: 74-82.
7. Elumalai EK, Ramachandran M, Thirumalai T and Vinothkumar P: Antibacterial activity of various leaf extracts of *Merremia emarginata*. Asian Pacific Journal of Tropical Biomedicine 2011; 1(5): 406-08.
8. Gandhi GR and Sasikumar P: Antidiabetic effect of *Merremia emarginata* Burm. F. in streptozotocin induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine 2012; 1: 1-6.
9. Prabhu TP, Pannerselvam P, Selvakumari S, Udhumansha U and Shantha A: Anticancer activity of *Merremia emarginata* (burm. f) against human cervical and breast carcinoma. International Journal of Research and Development in Pharmacy and Life Sciences 2012; 1(4): 189-92.
10. Babu AV, Rao RSC, Babu BH and Gottumukkala VS: Isolation and bioactivity of diacetyltetritol from *Merremia emarginata* (burm. f). Natural Products an Indian Journal 2013; 9(5): 201-08.
11. Prabhu TP, Panneerselvam P, Suresh R, Selvakumari S and Shantha A: Phytochemical analysis of ethanolic extract of *Merremia emaraginata* Burm. F by GC-MS. Research Journal of Pharmaceutical Biological and Chemical Sciences 2012; 3(4): 507-12.
12. Babu AV, Rao RSC, Kumar KG, Babu BH and Satyanaayana PVV: Biological activity of *Merremia emarginata* crude extracts in different solvents. Research Journal of Medicinal Plant 2009; 1: 1-6.
13. Prabhu TP, Panneerselvam P, Vijaykumar R, Atlee WC and Balasubramanian S: Anti-inflammatory, antiarthritis and analgesic effect of ethanolic extract of whole plant of *Merremia emarginata* Burm.f. Central European Journal of experimental Experimental Biology 2012; 1(3): 94-99.
14. Bonta RK: Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs). Journal of Pharmaceutical Analysis 2017; 7: 349-64.
15. Sahariah P, Bora J, Patar AK, Syiem D and Bhan S: Evaluation of antihyperglycemic and antioxidative effects of *Dillenia indica* L. using *in-vivo* approaches. International Journal of Pharmaceutical Sciences and Research 2018; 9(11): 4551-61.

How to cite this article:
Devadasu C, Prasad RY, Babu SP and Gananadhamu S: Biological screening and phytochemical investigation of *Merremia emarginata* leaves. Int J Pharm Sci & Res 2021; 12(1): 507-14. doi: 10.13040/IJPSR.0975-8232.12(1).507-14.