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BERGENIN - AN ACTIVE CONSTITUENT OF *RIVEA ORNATA* ROXB. AND ITS ANTIOXIDANT PROPERTY

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Keywords:

Rivea ornata Roxb, Phytochemical study, Antioxidant activity

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ABSTRACT: A simple TLC method has been developed for the simultaneous quantification of bergenin using HPTLC plate precoated with silica gel 60 F254. The method was developed in toluene: ethyl acetate: acetone (2:4:4 v/v) toluene: ethyl acetate: formic acid (4:6:1, v/v) and validated in terms of precision, repeatability, and accuracy. The isolated compounds were characterized using spectroanalytical techniques and found to be bergenin. The *in vitro* antioxidant activity of the isolated compound was determined. For the antioxidant potential, two standard analytical protocols, namely, DPPH radical scavenging activity (RSA) and Ferric reducing antioxidant power were adopted. The results showed that compound was found to be more potent antioxidant.

INTRODUCTION: Medicinal plants can be cultivated within a home or community garden, and many grow wildly.[1, 2] Some vegetables and fruits like berries, grapes, walnuts, olives, and foods like chocolate, wine, coffee and tea and popcorn, and some breakfast cereals contain large amounts of healthful antioxidant substances called polyphenols.

Bergenin is often included in thermogenic fat burners along with ingredients that stimulate norepinephrine release for its ability to enhance the breakdown of fat by this hormone. Bergenin may also be purchased by itself as an extract of Bergenia root.[3, 4, 5, 6, 7, 8]

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Bergenin containing extracts have long been used as a folk medicine in several parts of Asia. The molecular formula and the chemical structure of bergenin were confirmed by several spectroscopic methods and also by its synthesis [9]. Bergenin exhibits antihepatotoxic, antiulcerogenic, anti-HIV, antiarrhythmic, neuroprotective, anti-inflammatory and immunomodulatory properties [9].

Hyper physiological burden of free radical causes imbalance in homeostatic phenomenon between oxidants and antioxidants in the body. The imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases like arteriosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's and Parkinsonism. Therefore research in recent past have accumulated enormous evidence advocating enrichment of body system with antioxidants to correct vitiated homeostasis and prevent onset as well as treat the disease caused due to free radical and related oxidative

stress. Stress, smoking, drugs & diet generates excessive free radicals in human body.

Antioxidants have defined as the substance those in small quantities, able to prevent or greatly retard oxidation of easily oxidisable material such as fats. Antioxidants may exert their effect by different mechanisms such as suppressing the formation of active species by reducing hyperoxides (ROO) and H_2O_2 and also by sequestering metal ions scavenging active free radicals, repair and/or clearing damage.

MATERIAL AND METHOD:

Authentication and Collection of Plant: Dry plants of *Rivea ornata* were collected from the botanical garden of S.V. University, Tirupati in the month of May 2011 and its authentication was confirmed by Botanist, Dr. Madhava Chetty, S.V. University Tirupati. Herbarium of the plant has been deposited at Department of Pharmacognosy, B. Pharmacy College, Rampura, Kakanpura, Dist. Panchmahal, Gujarat, India for future reference.

Preparation of samples: Aerial parts of plant were used for Pharmacognostical studies. Aerial parts were collected, dried and powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies.

Proximate analysis: [10 - 15] Proximate analysis aids to set up certain standard for dried crude drugs in order to avoid batch-to-batch variation and also to judge their quality. Their studies also give an idea regarding the nature of phytoconstituents present **Table 2.** Proximate analysis of these crude drug powders was carried out using methods prescribed in the ayurvedic pharmacopoeia of India by subjecting them to various determinations like:

- **i.** Total Ash
- **ii.** Acid insoluble ash
- **iii.** Water soluble ash
- **iv.** Alcohol soluble extractive value
- **v.** Water soluble extractive value
- **vi.** Loss of moisture content

Determination of ash value: Ash values of powder of aerial part of *Rivea ornata Roxb.* were determined by the following method:

(a) Determination of total ash: 2 gm of accurately weighed powder was incinerated in a crucible at a temperature 500 - 600 ^oC in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

(b) Determination of acid insoluble ash: The total ash obtained above was boiled for 5min with 25 ml of 2M hydrochloric acid and filtered using an ash less filter paper to collect insoluble matter. The ash obtained was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was calculated with reference to the air-dried powered drug (60#).

(c) Determination of water soluble ash: The total ash was boiled for 5min with 25 ml of water and insoluble matter collected on an ash-less filter paper washed with hot water and ignited for 15 min at a temperature not exceeded 450 ^oC in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powered drug.

Determination of extractive values: Extractive values of powder of aerial parts of *Rivea ornata* was determined by the following methods:

(a) Determination of alcohol soluble extractive value: 4g of the air-dried powdered material were macerated with 100 ml of alcohol in a closed flask for 24 h, shaking frequently at an interval of 6 h. It was then allowed to stand for 18 h and filtered rapidly to prevent any loss during evaporation. 25 ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105 °C to a constant weight. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

(b) Determination of water soluble extractive value: 4g of the air-dried powdered material were soaked in 100ml of water in a closed flask for 1h with frequently shaking. It was then boiled gently for 1 h on water bath; cooled and weighed and readjusted the weight. 25 ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105 °C to a constant weight. The percentage of water-soluble extractive was calculated with reference to the air-dried powered drug (60#).

Determination of moisture content: Placed about 100 gm aerial part of *Rivea ornata* Roxb. after accurately weighing in a tarred evaporating dish. After placing the above said amount of the drug in the tarred evaporating dish, dried at 105 ^{0}C for 5 hours, and weighed. Continued the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 50mins.and cooling for 30 minutes in a desiccator, showed not more than 0.01gm difference.

Phytochemical Studies:

Preliminary Phytoprofiles: [16, 17] Successive solvent extraction: 10g of the air-dried powdered plant material was successively extracted with the following solvents of increasing polarity in a soxhlet apparatus.

a) Petroleum ether (60 - 80 ºC)
b) Ethyl acetate
c) Chloroform
d) Methanol
e) Water

All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven at 50 ^{0}C. Each time before extracting with the next solvent, the marc was dried in an air oven below at 50 ^{0}C. The marc was finally macerated with water for 24 hours to obtain the aqueous extract.

The completion of the extraction was confirmed by evaporating a few drops of extract from the thimble on watch glass to observe that no residue remained after evaporation of the solvent. The liquid extracts obtained with different solvents were collected. The consistency, odour, colour, appearance of the extracts and their percentage yield were noted. The extracts were then subjected to various qualitative test using reported methods, to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, sterols and terpenoids, cardiac glycosides, coumarins, carotenoids, tannins, phenolic compounds, fixed oils and fats *etc*. **Table 4.**

Qualitative chemical identification of *Rivea ornate*: [16 - 20] The extracts were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolic and tannins, phytosterols, fixed oils and fats, proteins amino acids, flavonoids, saponins, etc. using reported methods.

Evaluation of antioxidant activity of *Rivea ornata* Roxb.:

Instruments: UV spectrophotometer (Shimadzu-UV-1601), Centrifuge Machine (Eltek-research centrifuge-TC-4100D).

Chemicals: All chemicals used for the study are purchased from SD-fine chemicals; India and all other reagent used were of analytical grade.

DPPH radical scavenging activity: [21 - 23] Product extract and standard ascorbic acid solution (0.1 ml) of different concentrations *viz*. 10, 20, 40, 60, 80, 100µg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition activity was calculated from [(A0-A1)/A0] ×100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations **Table 5** and **Fig. 5**

Ferric Reducing Power determination: [21 - 23] Different concentrations of plant extract and standard ascorbic acid solution *viz.* 10, 20, 40, 60, 80, 100 µg/ml in 1ml of methanol were mixed with phosphate buffer (2.5ml, 0.2M pH 6.6) and potassium ferric cyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of tricholoro acetic acid (10%) was added to the mixture, which was then centrifuged at 3000 g (rpm) for 10 min at room temperature. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride ($FeCl_3$) (0.5 ml, 0.1%) and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700nm. All the tests were performed in triplicate and the graph was

plotted with the average of three observations. **Table 6** and **Fig. 6**

RESULT AND DISCUSSION:

Identification and Authentication of *Rivea ornata* Roxb: Fresh and dry plants of *Rivea ornata* were collected from, Tirupati the month of May and its authentication was confirmed by Botanist, Dr. Madhava Chetty, S.V. University Tirupati. Herbarium of the plant has been deposited at Department of Pharmacognosy, B. Pharmacy College, Rampura, Kakanpura, Panchmahal, Gujarat, India for future reference. VJS/SD-35. The plant was further subjected to morphological and microscopically examination to access the purity of the procured plant drug and various diagnostic features were recorded for the purpose of identification.

Proximate Analysis:

TABLE 1: STUDY OF DIFFERENT PARAMETERS OBTAINED FROM PROXIMATE ANALYSIS OF AERIAL PARTS OF *RIVEA ORNATA* ROXB.

S. no.	Determination	Percentage w/w
1.	Total Ash	15.45
2.	Acid insoluble Ash	6.63
3.	Water Soluble Ash	3.24
4.	Alcohol Soluble Extractive value	4.69
5.	Water soluble Extractive value	7.15
6	Moisture content	70.86

Phytochemical Studies:

Preliminary Phytoprofile: The percentage of different chemical constituents in the crude drug can be detected by subjecting them to successive extraction using solvents in the order of increasing polarity. The extract obtained were then dried completely and kept in vacuum desiccators. They were then subjected to qualitative chemical tests in order to detect the various chemical constituents present in them.

TABLE 2: PRELIMINARY PHYTOPROFILE OF AERIAL PARTS OF *RIVEA ORNATA ROXB*

S. no.	Solvent	Color and consistency after drying	Average value (%w/w)
1.	Petroleum ether (60 - 80ºC)	Yellowish, solid mass	2.50
2.	Ethyl acetate	Greenish, sticky mass	3.21
3.	Chloroform	Greenish, sticky mass	2.13
4.	Methanol	Greenish yellow, sticky mass	6.21
5.	Water	Dark Brown solid mass	9.61

Tests for preliminary Phytochemical Screening of powder of aerial part of *Rivea ornata Roxb.*:

- Qualitative chemical examination of various successive extracts of powder indicated the presence of carbohydrates, steroids, Triterpenoid glycosides, alkaloid, phytosterols, steroids, mucilage.

Phytosterols were detected by Libermann Burchard test and salkowaski reaction, carbohydrates by molisch's, Fehling's and Benedict's test, saponin by foam test, flavonoids by shinoda test, tannins and phenolics by Lead acetate test.

TABLE 3: TEST FOR PRELIMINARY PHYTOCHEMICAL SCREENING OF AERIAL PARTS OF *RIVEA ORNATA* ROXB.

S. no.	Tests of Phytoconstituents	P. ether extract	Ethyl acetate extract	Chloroform extract	Methanol Extract	Water Extract
1	Tests for alkaloids					
	a) Mayer's reagent	*	*	+ve	+ve	+ve
	b) Dragendorff's reagent	*	*	+ve	+ve	+ve
	c) Hager's reagent	*	*	+ve	+ve	+ve
	d) Wagner's reagent	*	*	+ve	+ve	+ve
2	Tests for flavonoids					
	a) Shinoda test	*	*	*	-ve	-ve
	b) Fluorescence test	*	*	*	-ve	-ve
	c) $FeCl_3$ test	*	*	*	-ve	-ve
	d) Lead acetate test	*	*	*	-ve	-ve
3	Tests for saponins					
	a) Froth test	*	*	*	+ve	+ve
	b) Hemolytic zone	*	*	*	+ve	+ve
4	Tests for carbohydrates					
	a) Molisch's test	*	*	*	+ve	+ve
	b) Fehling's solution test	*	*	*	+ve	+ve
	c) Benedict's test:	*	*	*	+ve	+ve

5	Tests for cardiac glycoside					
	a) Legal's test	*	*	*	-ve	-ve
	b) Keller Killiani's test	*	*	*	-ve	-ve
	c) Baljet test	*	*	*	-ve	-ve
6	Tests for fixed oil and fat					
	a) Spot test	+ve	+ve	*	*	*
	b) Saponification test	+ve	+ve	*	*	*
7	Tests for sterols and triterpenoids					
	a) Libermann-burchard`s test	+ve	*	*	*	*
	b) Salkowski reaction	+ve	*	*	*	*
8	Tests for anthraquinone glycosides					
	a) Borntrager's test	*	*	*	-ve	-ve
	b) Modifying borntrager's test	*	*	*	-ve	-ve
9	Tests for phenolic compounds					
	a) Test with $FeCl_3$	*	*	*	+ve	+ve
	b) Test with folin-ciocalteu reagent	*	*	*	+ve	+ve
10	Tests for coumarins					
	a) With ammonia	*	*	*	-ve	-ve
	b) With hydroxylamine hydrochloride	*	*	*	-ve	-ve
11	Tests for tannins					
	a) Test with gelatin	*	*	*	+ve	+ve
	b) Reaction with lead acetate	*	*	*	+ve	+ve

Isolation and identification of phenolic compound from the arial parts of plant *Rivea ornate*: The methanol extract of *Rivea ornata* prepared by successive solvent extraction technique was used to isolate bergenin.

TLC Profile of Test solution of *Rivea ornata Roxb.*: 10µl of the methanol extract was spotted on TLC plate. It was developed in toluene:ethyl acetate:acetone (2:4:4) mobile phase. The plate was observed under UV light at 254 nm and after spraying with vaniline sulphuric acid and heating at 100˚C till the colored bands appear.

Identification of the isolated phenolic compound: The compounds C1 isolated at R_f – 0.74 showed positive chemical tests for phenolic compound (vaniline sulphuric acid). Melting point of C2 was 232-234°C. Its structure was confirmed by different analytical techniques like UV-spectroscopy, IR-spectroscopy, LC-MS spectroscopy and NMR-spectroscopy. For final confirmation UV spectra of compound C1 was taken with standard bergenin.

UV- Spectroscopy: The ultra violet spectra of compounds C1 was taken in methanol. The UV-spectra of compounds C1 was shown in **Fig. 1** respectively.

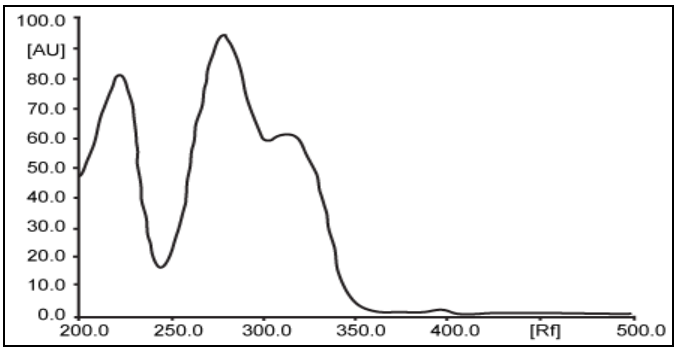


FIG. 1: ULTRAVIOLET SPECTRUM OF COMPOUND C1

Interpretation:

Sample ID	λ_{max}
Compound-C1	277 nm

MS spectroscopy: The MS spectra of compounds C1 was taken. The MS spectra of compounds C1 is shown in (**Fig. 2**, **Table 4**) respectively

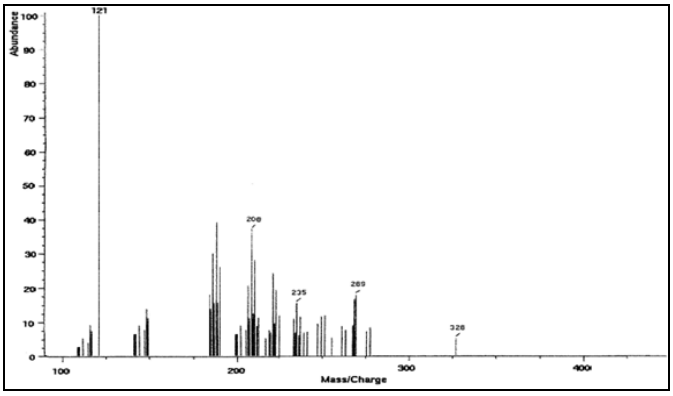


FIG. 2: MS SPECTRUM OF COMPOUND C1

TABLE 4: INTERPRETATION

Sample ID	*m/z*	Fragments
Compound- C1	121	[MH]
	208	[MH-H_2O]
	235	[$C_{16}H_{15}N_2O$]
	269	[$C_{15}H_{15}N_2$]
	326	

The signals of the mass spectrum and their interpretation are consistent with the molecular formula of compound.

IR-spectroscopy: The Infrared spectrum of compound C1 was taken. The IR-spectrum of compound C1 is shown in **Fig. 3** and **Table 5**.

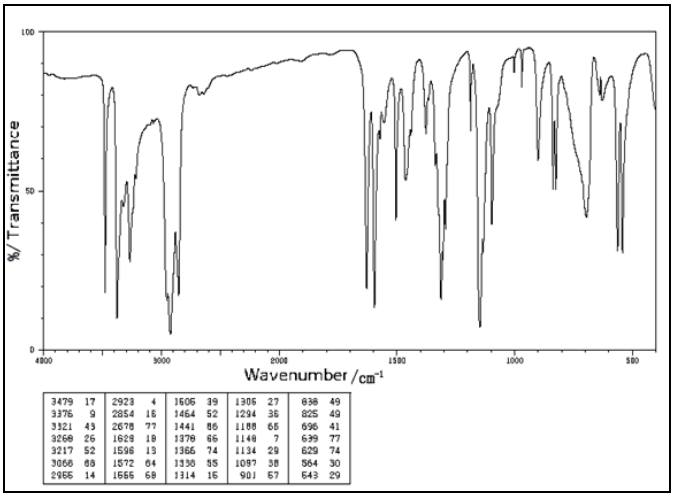


FIG. 3: INFRARED SPECTRUM OF COMPOUND C1

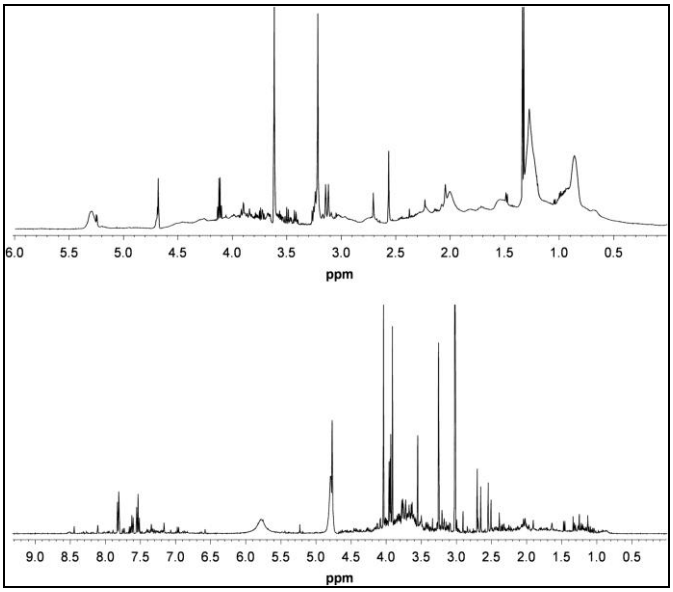
Interpretation:

From the data obtained by FTIR, it could be concluded that following functional groups are present.

TABLE 5: DATA FOR IR SPECTRUM OF COMPOUND C1

Functional Group	Sample Peak (cm^{-1})	Standard Peak (cm^{-1})
O-H stretch	3396	3200-2500
C-H stretch (alkanes)	2936	3200-2500
C=O stretch	1710	1735-1750
C=C stretch	1634	1620-1680
C=C stretch	1610	1620-1680
C-O-C	1363	1200-1320
C-C stretch	1076	1000-1260
C-C(=O)-O	1022	1000-1300

NMR - Spectroscopy: The 1H-NMR and 13C-NMR spectra of compound C1 were taken. The 1H-NMR and 13C NMR spectra of compound C1 are shown in **Fig. 4** and **Table 6** respectively.



H-NMR of Compound 1

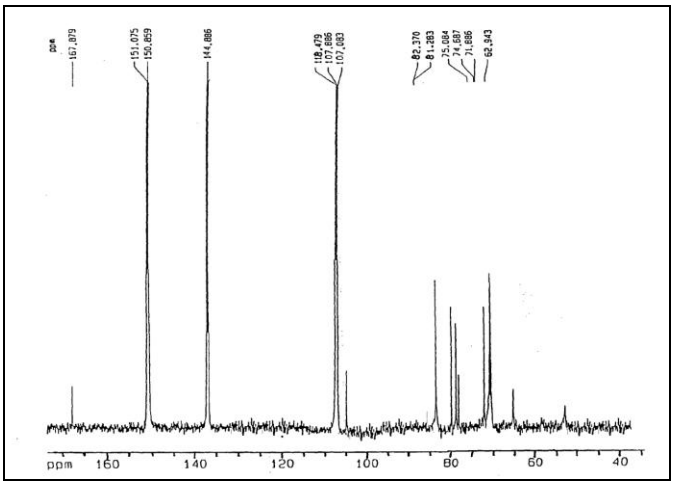


FIG. 4: H-NMR AND C-NMR OF COMPOUND 1

TABLE 6: ^{1}H NMR AND ^{13}C NMR SPECTRAL DATA OF COMPOUND 1 (CD_3OD)

Position	^{1}H NMR (40MHz)	^{13}C NMR (100 MHz)
1		118.6
2		107.6
3		144.9
4		151.4
5		150.8
6	7.13 (1H, s)	107.5
7		167.8
1′	4.86 (1H, d, *J* = 10.3 Hz)	74.6
2′	3.78 (1H, m)	75.8
3′	3.95 (1H, m)	81.4
4′	3.44 (1H, m)	71.8
5′	3.62 (1H, m)	82.8
6′	3.68 (1H, m)	62.7
	4.00 (1H, m)	
OCH_3	3.81 (3H, s)	

Compound C1 was isolated as white crystals. UV_{max} (MeOH): 275 nm; IR bands (KBr): 3425, 2885, 2724, 1702, 1614, 1528, 1464, 1421, 1375, 1341, 1233, 1093 and 1046 cm^{-1}. The mass spectral data of the compound gave a molecular formula $C_{14}H_{16}O_9$, *m/z* 328 for M^+. ^1H NMR and C NMR was shown above.

From the spectral data it was concluded that the compound C1 was Bergenin

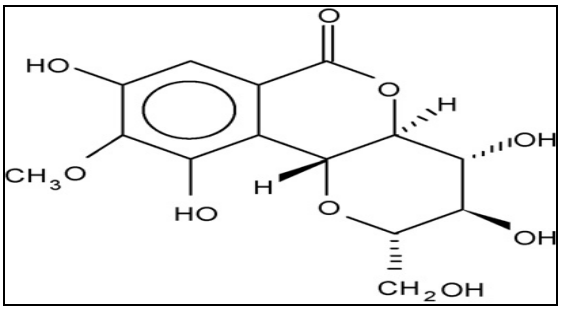
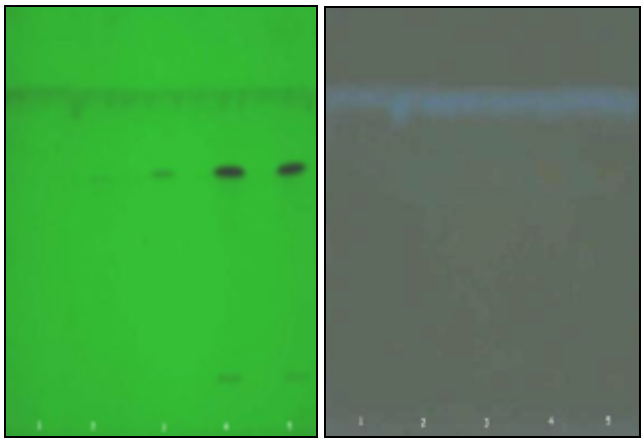


FIG. 5: STRUCTURE OF BERGENIN

HPTLC finger-printing and quantitative determination of isolated Bergenin: CAMAG TLC scanner 3 and LINOMAT-V densitometry evaluation system with WINCAT software was used for scanning of thin layer chromatogram objects in reflectance or transmission mode by absorbance or by fluorescence at 254 or 365 nm, respectively.

R_f value of sample was evaluated using following formula. R_f = Distance traveled by sample from baseline Distance traveled by solvent from baseline Calibration of Bergenin.

Different concentration (1µg/ml-5µg/ml) using micro syringe from the standard solution of 1000 mcg/ml were spotted as sharp band of 5 mm width on precoated silica gel aluminum plate 60F254, (10cm x 10cm) using toluene:ethyl acetate:acetone (2:4:4).



Under UV 254 nm **Under UV 365 nm**

FIG. 6: HPTLC PLATS OF BERGENIN

Track-1: 1 µg/ml of Standard bergenin. Track-2: 2 µg/ml of Standard bergenin. Track-3: 3 µg/ml of Standard bergenin. Track-4: 4 µg/ml of Standard bergenin. Track-5: 5 µg/ml of Standard bergenin

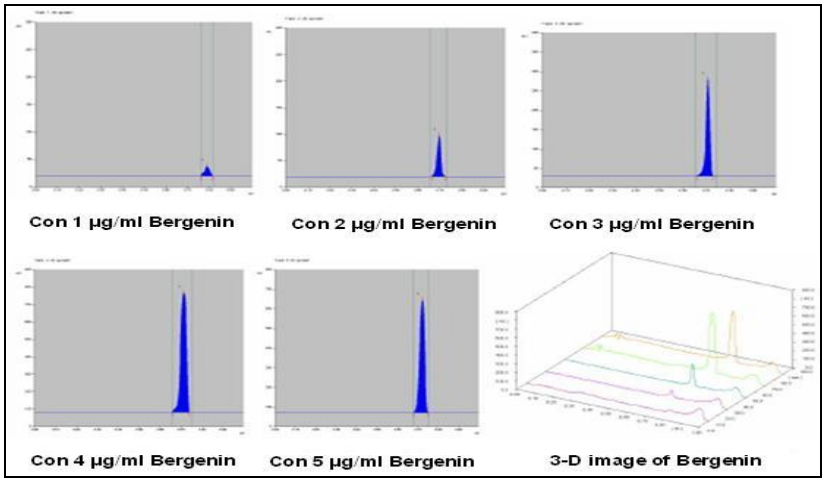


FIG. 7: DENSITOMETRY CHROMATOGRAM OF BERGENIN STANDARD

Concentration (µg/ml)	Area
1	529.2
2	1783.3
3	4192.2
4	5284.2
5	7254.3

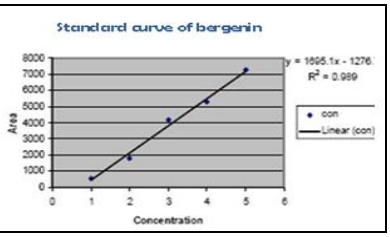
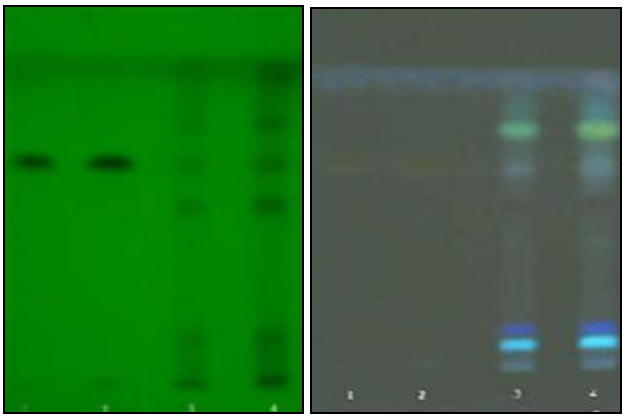


FIG. 8: CALIBRATION CURVE OF STANDARD BERGENIN

The peak areas of bergenin for (1µg/ml-5µg/ml) concentration were recorded. Calibration curve was prepared by plotting peak areas of bergenin against concentration (**Fig. 8**). The results of linearity range and correlation coefficient showed that within the concentration (1µg/ml-5µg/ml) range indicated; there was good correlation between peak area and the corresponding concentration of bergenin. The best fitting line equation was y = 1695.1X - 1276.7.

HPTLC of Bergenin with *Rivea ornata* Roxb:

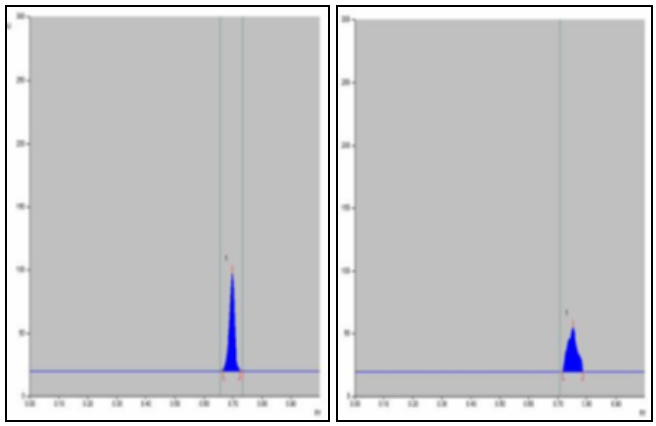


1 2 3 4 1 2 3 4

A- Under UV 254 nm B-Under UV 365 nm

FIG. 9: HPTLC PLATES OF *RIVEA ORNATA ROXB.*

Track-1: 2 µg/ml of standard bergenin. Track-2: 4 µg/ml of standard bergenin. Track-3: 2 µg/ml of Methanol extract of *Rivea ornata* Roxb. Track-4: 4 µg/ml of Methanol extract of *Rivea ornata* Roxb.



3D- image of the extracts *Rivea ornata* Roxb

FIG. 10: DENSITOMETRY CHROMATOGRAM OF *RIVEA ORNATA* ROXB.

TABLE 7: HPTLC FOR BERGENIN IN *RIVEA ORNATA* ROXB.

Track	Peak	Start R_f	Start Ht.	Max R_f	Max Ht.	End R_f	Area	Bergenin mcg/ml
Bergenin	1	0.71	33.2	0.74	43.4	0.76	867.4	---
Methanolic extract *Rivea ornata Roxb.*	1	0.72	0.6	0.74	19.1	0.76	1470.5	0.331

Silica gel TLC plate as stationary phase and toluene: ethyl acetate: acetone (2:4:4) as mobile phase gives good separation of Bergenin at R_f - 0.74. The HPTLC photographed chromplate is shown in (**Fig. 6**). The detector response/calibration curve of Bergenin was found to be linear dependent on the concentration against area. The best fitting line equation was y = 1695.1X – 1276.7. Correlation coefficient 0.989 indicates good linearity between concentration and peak area in (**Fig. 8**). The concentration of Bergenin in the methanolic extract of dried leaves powder of *Rivea ornata* Roxb. by proposed HPTLC method was found to be 0.331mcg/ml. The identity of the Bergenin band in the sample extract solution was confirmed by overlaying/superimposing the UV absorption spectrum of the sample with that from the reference standard of bergenin, using the Camag TLC scanner 3 (**Fig. 9, 10** and **Table 7**).

DPPH Method:

TABLE 8: 1,1-DIPHENYL-2-PICRYL HYDRAZYL (DPPH) RADICALS SCAVENGING ACTIVITY

Sr. no.	Concentration µg/ml	% inhibition	
		Alcoholic extract	Ascorbic acid
1	0	0	0
2	10	43.84	45.56
3	20	50.2	55.5
4	40	58.5	60.33
5	60	60.59	47.65
6	80	61.96	70.22
7	100	65	75.15
8	IC_{50} value	21.2	11.33

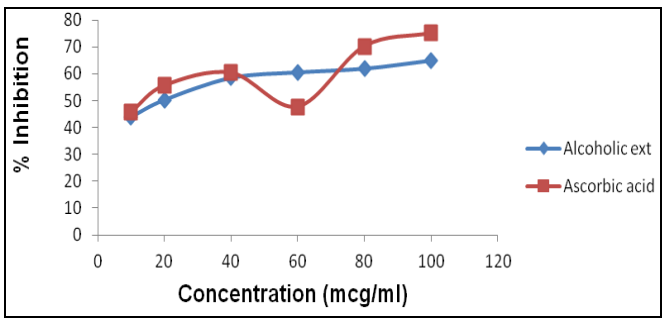


FIG. 11: 1,1-DIPHENYL-2-PICRYL HYDRAZYL (DPPH) RADICALS SCAVENGING ACTIVITY %INHIBITION V_S CONCENTRATION

Antioxidant activity of the plant *Rivea ornata* Roxb.: The antioxidant activity of the alcoholic extract of the plant *Rivea ornata* Roxb. was carried out by *in vitro* antioxidant models. In the models tested, the antioxidant activity of the formulation was studied in relation to Ascorbic acid, a known antioxidant.

Result indicated the significant decrease in the concentration of DPPH radicals due to the scavenging ability of Alcohol extract of plant *Rivea ornata* Roxb. and Ascorbic acid, as a reference standard. Maximum inhibition of extract and Ascorbic acid was exhibited 65.0% and 75.15% inhibition respectively in 100µg/ml. The IC_{50} values in DPPH radical scavenging model were 11.33µg/ml and 21.2 for Ascorbic acid and alcohol extract of plant *Rivea ornata* Roxb. respectively.

Ferric reducing antioxidant power:

TABLE 8: FERRIC REDUCING ANTIOXIDANT ACTIVITY

Ferric reducing power		
Concentration (mcg/ml)	Absorbance	
	Ascorbic acid	Alcoholic extract
0	0	0
10	0.05	0.024
20	0.08	0.048
40	0.11	0.079
60	0.152	0.12
80	0.195	0.155
100	0.252	0.195

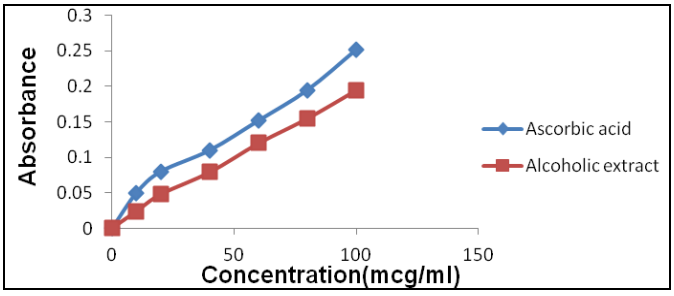


FIG. 12: FERRIC REDUCING ANTIOXIDANT ACTIVITY AB. *vs* CONC.

Result illustrates that Alcoholic extract of the plant *Rivea ornata* Roxb. had ferric reducing capacity and also comparable to Ascorbic acid.

CONCLUSION: *Rivea ornata* Roxb. (syn. Phang), family Convolvulaceae, is a woody climber, occurring in south India in Tripura. *Rivea ornata Roxb.* leaves contain total ash (13.80 %), acid insoluble ash (7.52 %), water soluble ash (6.21 %), alcohol soluble extractive value (6.58 %), water soluble extractive value (7.69 %) and moisture content (82.65 %). Petroleum ether ((60 - 80 ºC) extract of *Rivea ornata Roxb.* was yellowish mass (2.53 %w/w), Toluene extract was green sticky mass (3.12 %w/w), Chloroform extract was greenish yellow sticky mass (2.13 %w/w), Methanol extract was greenish brown sticky mass (3.21 %w/w) and Water extract was reddish brown sticky mass (8.24 %w/w).

Compound C2 was isolated as white crystals. UV_{max} (MeOH): 277 nm; IR bands (KBr): 3425, 2885, 2724, 1702, 1614, 1528, 1464, 1421, 1375, 1341, 1233, 1093 and 1046 cm^{-1}. The mass spectral data of the compound gave a molecular formula C14 H16O9, m/z 328 for M+.1H NMR (DMSO-d6, 400 MHz): δ 6.48 (m, arom., H-7), 5.68 (1H, d, H-10b), 4.99 (1H, dd, H-4a), 3.99 (1H, dd, H-4), 3.80 (2H, d, H-11), 3.76 (3H, s, H-12), 3.60 (1H, m, H-2), 3.49 (1H, dd, H-3). 13C NMR (DMSO- d6, 100 MHz): δ 60.0 (C-12), 61.2 (C-11), 70.8 (C-3), 72.2 (C-10b), 73.8 (C-4), 79.9 (C-4a), 81.8 (C-2), 109.6 (C-7), 116.1 (C-10a), 118.2 (C-6a), 140.7 (C-9), 148.2 (C-10), 151.1 (C-8), 163.5 (C-6). From the spectral data it was concluded that the compound C1 was Bergenin.

Qualitative chemical examination of various extracts of *Rivea ornata Roxb.* was carried out which revealed presence of phytoconstituents like carbohydrates, phytosterols, phenolic compounds, alkaloid, triterpenoids, fixed oil and tannins.

The reducing power of *Rivea ornata* Roxb. extracts increased steadily with increasing concentrations and varied significantly with different concentrations. The methanol and ethyl acetate extracts appeared to possess the highest significant reducing activity among the extracts. The stronger reducing power in the methanol and ethyl acetate extracts was probably due to the concentration of antioxidant compounds like flavonoids and phenolics in the extract. In conclusion, antioxidant study of *Rivea ornata* Roxb. suggested that *Rivea ornata* Roxb. is a potential source of natural antioxidants. However, further investigations on *in vivo* antioxidant activities are highly recommended. It is also needed to determine phytoconstituents, which are responsible for the antioxidant activity.

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CONFLICTS OF INTEREST: Nil.

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