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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of antioxidant activity and HPTLC analysis of *Bridelia retusa* bark

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ABSTRACT
The aim of present study was to screen bark Extracts of Bridelia retusa for in vitro Antioxidant activities to find the new sources of antioxidants. HPTLC analysis was performed in butanolic extract to determine Gallic acid and Catechin. Antioxidant activity of extracts was determined byusing DPPH, ABTS assay method. Higher antioxidant potentiated was observed by in both, DPPH and ABTS assay method by butanolic extract. The finding indicates the Gallic acid and Catechin may have antioxidant potential.
Keywords: *Bridelia retusa, DPPH, BRBB, HPTLC*

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INTRODUCTION

The various medicinal plant extracts and their secondary metabolites have served as antioxidants to protect against various diseases from long ago [1]. High performance thin layer chromatography (HPTLC) is widely used to established reference fingerprints of the plant extract it is powerful tool for quality control of raw plant material and herbal formulations [2, 3, 4]*Bridelia retusa* (Euphorbiaceae), A small or moderate sized tree, spinus when young and has gray bark. Leaves are used in Ayurvedic formulation for the treatment of urinary tract infections and bark is given orally to women to develop sterility and as contraceptive[5].The root and the bark are valuable astringent. Stem bark and roots were used for rheumatism and as astringent agents [6].The paste of leaves of *Bridelia retusa*, *Curculigo orchioides* and oil of castor, coconut and gingelly applied externally to cure wounds [7].The *Bridelia retusa* in combination with other plants used traditionally to cure hepatitis[8]. The *Bridelia retusa* bark also used to cure dysentery[9].*Bridelia retusa* Bark and leaves extract also shows hepatoprotective activity on various animal models.[10, 11]

MATERIAL AND METHODS

Plant and Material

Bridelia retusa bark was collected from Western Ghat of Maharashtra and authenticated by J. Jayanthi scientist, Botanical Survey of India, Pune and herbarium was (Voucher No. BRIRVIGI) deposited in BSI Pune.

Preparation of Extracts:

The fresh bark was cleaned, shade, dried and then powdered. Firstly the dried coarse powder of bark was defatted with petroleum ether later on it was subjected to successive extraction in a soxhlet apparatus using solvents chloroform, butanol and 70% acetone. Chloroform extract is named as BRBC extract. Butanolic extract named as BRBB extract and Acetone extract names as BRAB extract.

HPTLC analysis of *Bridelia retusa* extract

TLC Conditions.:

TLC plate consists of 20 × 10 cm, precoatedwith silica gel 60 F254 TLC plates (E.Merck) (0.2mmthickness) with aluminum sheet support. The spotting devicewas a CAMAG Linomat V Automatic Sample Spotter (CamagMuttenz, Switzerland); the syringe, 100 μL (fromHamilton); the developing chamber was a CAMAG glasstwin trough chamber (20 × 10 cm).
Mobile phase was Petroleum ether: Ethyl acetate: Formic acid (4:6:0.1) Saturation time for mobile phase was 2 hours.

Procedure:
The butanol extract of bark of *B. retusa*was applied on TLC plate and the plate was developed in Petroleum ether: Ethyl acetate: Formic acid (4:6:0.1) solvent system to a distance of 8 cm. The plates were dried at room temperature in air. The plate was scanned at 254nm.

Quantification of Gallic acid and catechin in butanol Extract Using HPTLC Method

Standard Stock Solution.
A solution of Gallic (10 and 20 μg/mL) acid and catechin (10 and 20 μg/mL) was prepared in methanol.

Sample Preparation:
ButanolicExtract (BRBB extract). Stock solution of sample 100 and 200 µg/mL of extract was prepared in butanol.

After each chromatography, the percentage content of each marker in respective plant extract was calculated as per the following formula.

$$\% \text{ content } = \frac{\text{AUC of Sample X Conc of Std. X \% purity}}{AUC of Std X Conc. of sample}$$

In *Vitro* antioxidant study:

DPPH radical scavenging assay
The effect of the extracts on DPPH radical was estimated using following method. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02–0.1 mg of the extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity \%} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract /standard. [12]

ABTS radical scavenging assay
To determine ABTS radical scavenging assay, the following method. was adopted. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium per sulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of ascorbic acid and percentage inhibition calculated as

$$\text{ABTS radical scavenging activity \%} = \frac{\text{Abs control} - \text{Abssample}}{\text{Abs control}} \times 100$$

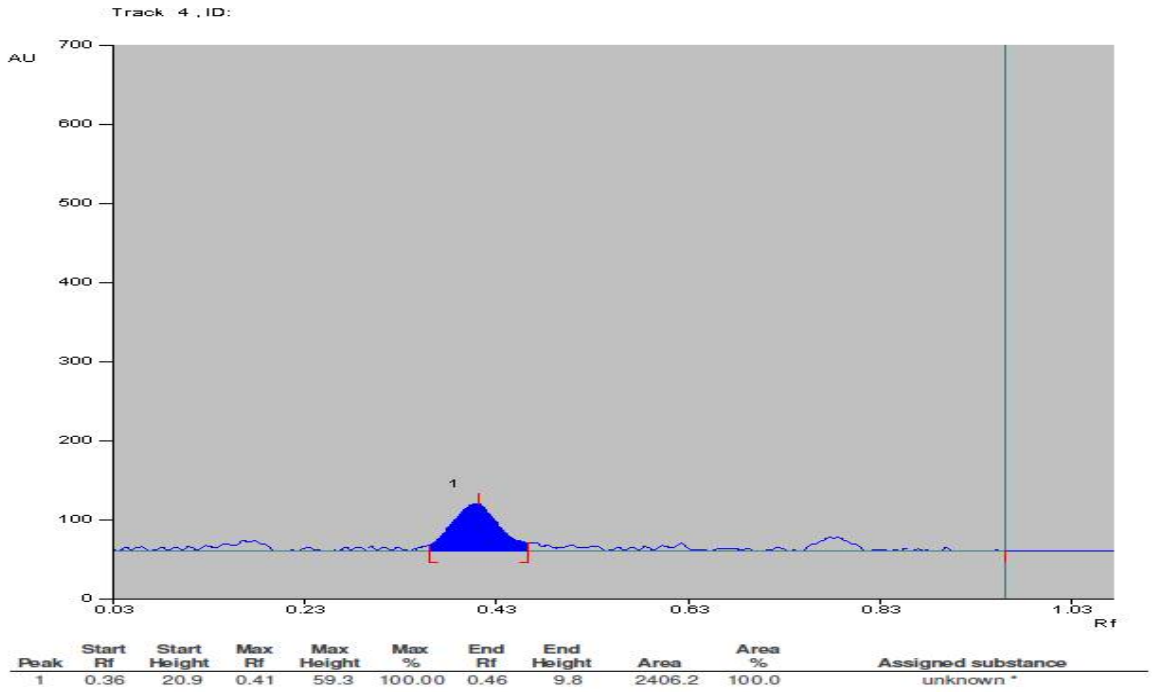
where Abs control is the absorbance of ABTS radical + methanol; Absorbance of sample is the absorbance of ABTS radical + sample extract /standard.[13]

RESULT AND DISCUSSIONS

HPTLC Analysis:
The percentage (W/W) amount of gallic acid was found to 6.21% and catechin 3.24% in butanol extract of *B. retusa*. (Tables 1) with 3784.2 and AUC 780.4 respectively for Gallic acid and Catechin respectively.

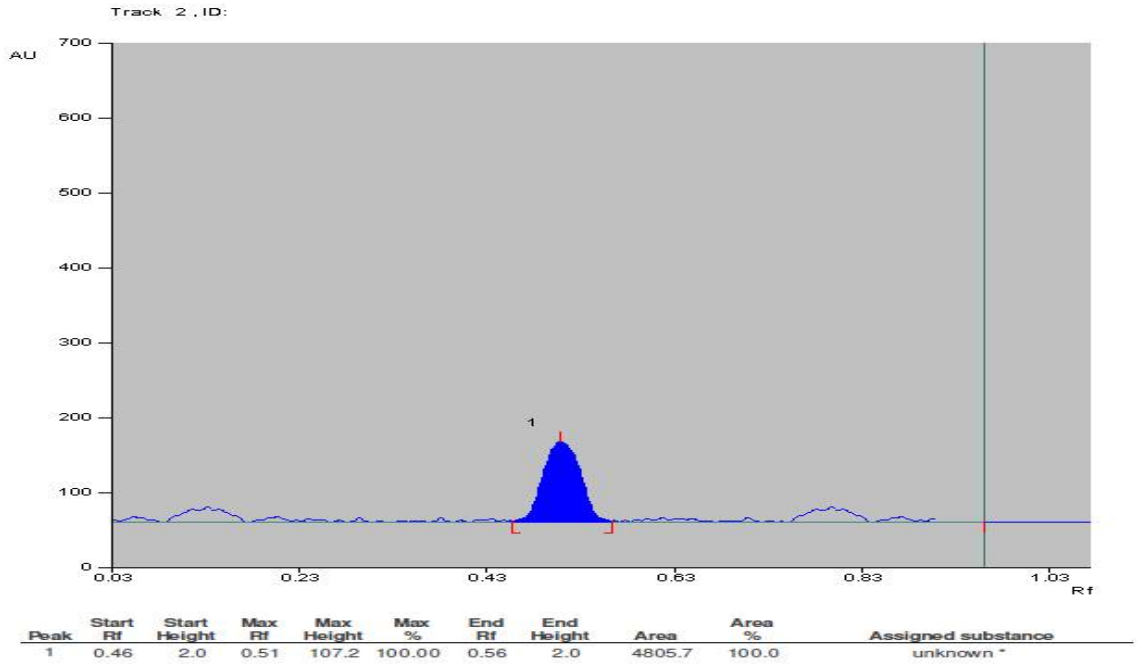
Table.1. Estimation of Gallic acid and Catechin in BRBB extract.

Extract / marker	Rf	AUC	%Content
catechin (20 µg/ml)	0.41	2406.2	100
BRBB (200 µg/ml)	0.41	780.4	3.24
Gallic acid (20µg/ml)	0.51	4805.7	100
BRBB(200 µg/ml)	0.50	3784.2	6.21



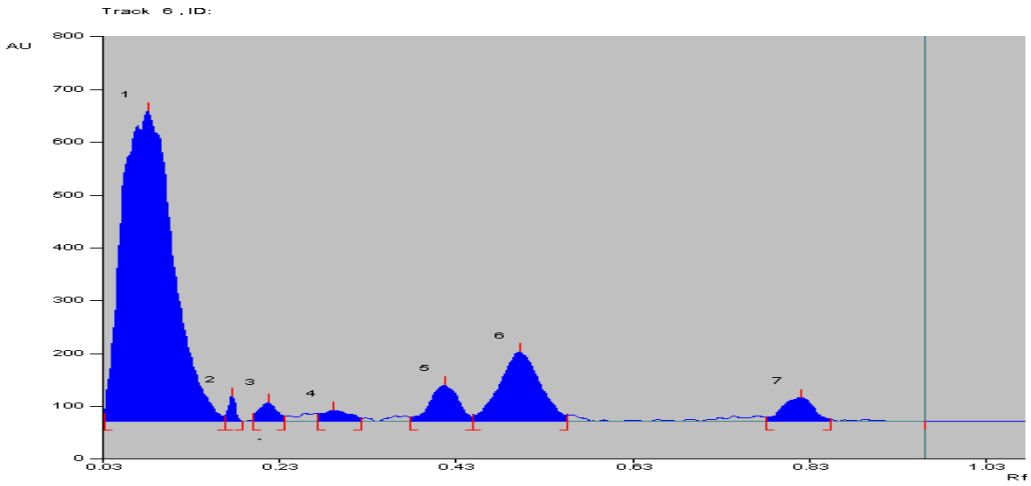
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.36	20.9	0.41	59.3	100.00	0.46	9.8	2406.2	100.0	unknown *

Fig.1.HPTLC Chromatogram of Catechin standard.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.46	2.0	0.51	107.2	100.00	0.56	2.0	4805.7	100.0	unknown *

Fig.2. HPTLC Chromatogram of Gallic acid standard.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	13.2	0.08	678.9	65.51	0.17	11.20	19245.8	81.47	Unknown
2	0.17	11.2	0.18	51.3	4.23	0.19	42.11	89.4	0.38	Unknown*
3	0.20	14.3	0.22	48.2	3.42	0.24	11.20	115.1	0.49	Unknown*
4	0.28	14.6	0.31	22.6	2.72	0.34	7.50	127.5	0.54	Unknown*
5	0.38	12.5	0.41	83.4	6.87	0.45	14.60	780.4	3.30	Unknown*
6	0.45	14.6	0.50	145.7	13.12	0.55	11.20	3784.2	16.02	Unknown*
7	0.78	8.9	0.82	45.8	4.13	0.85	4.10	278.4	1.18	Unknown*

Fig.3. HPTLC Chromatogram of BRBB extract.

DPPH radical scavenging activity:

The dose-response curve of DPPH radical scavenging activity of the chloroform, butanol and acetone extracts bark of *Bridelia retusa*, on compared with ascorbic acid. It was observed that butanol extract of bark of *Bridelia retusa* had higher activity than that of the chloroform and acetone extract. The IC_{50} (µg/ml) was 199.26±8.74, 124.17±6.98, 142.89±4.32and 69.54±4.51for BRBC, BRBB, BRBA and Ascorbic acid respectively, Could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.(Table-2)

Table.2Antioxidant effect (IC_{50}) on free DPPH radicals of *B. retusa* bark extracts

Extracts (µg/ml)	% inhibition				Ascorbic acid (µg/ml)
	BRBC	BRBB	BRBA	As. acid	
50	18.18 ±0.98	33.43±2.69	35.77±2.66	28.01±1.59	20
100	28.29 ±1.54	51.02±5.11	37.09±1.68	38.12±3.98	40
150	46.18 ±2.39	55.86±4.64	52.63±3.54	46.48±2.38	60
200	47.21 ±2.17	63.78±5.67	65.98±5.97	54.40±4.76	80
250	51.46 ±2.54	66.42±6.11	66.27±4.57	62.61±3.25	100
300	77.85 ±6.29	75.95 ±6.98	74.78±6.54		
IC_{50} (µg/ml)	199.26±8.74	124.17±6.98	142.89±4.32	69.54±4.51	

ABTS radical scavenging activity:

The chloroform, butanol and acetone extracts of bark of *Bridelia retusa*were effective scavengers of the ABTS radical and this activity was comparable to that of ascorbic acid. The IC_{50} (µg/ml) was 143.28±4.64, 70.88±3.98, 81.582±4.27and 37.65±1.95for BRBC, BRBB, BRBA and Ascorbic acid respectively,

Table.3. Antioxidant effect (IC_{50}) on free ABTS radicals of *B. retusa* bark extracts.

Extracts (µg/ml)	% inhibition				As. Acid (µg/ml)
	BRBC	BRBB	BRBA	As. acid	
50	24.32±1.68	46.54±3.14	42.15±2.14	41.24±1.13	20
100	44.51±3.14	56.42±4.32	52.31±3.33	51.14±2.12	40
150	57.88±5.11	67.51±6.21	63.46±4.25	61.65±2.41	60
200	62.12±4.59	73.14±5.46	69.54±4.12	67.34±1.89	80
250	73.44±4.97	75.18±6.47	74.44±5.78	71.44±3.14	100
300	75.41±6.21	78.21±8.21	76.44±5.34		
IC_{50} (µg/ml)	143.28±4.64	70.88±3.98	81.582±4.27	37.65±1.95	

The effect of antioxidant on DPPH and ABTS radical scavenging is thought to be due to their hydrogen donating ability. The results obtained in this study suggest that all extracts from *Bridelia retusa* showed radical Scavenging activity by their hydrogen donating ability. HPTLC analysis of Butanolic extract of *Bridelia retusa* barks extracts show the presence of Gallic acid and Catechin. Antioxidant activity of *Bridelia retus*a may be due the presence of Gallic acid, Catechin.[14]

CONCLUSION

The invitro antioxidant study indicate that Bridelia retusa Bark is a significant source of Antioxidant which could help to present the progression of various diseases caused by free radical. HPTLC analysis of Butanolic extracts shows the presence of Gallic acid and Catechin may responsible for antioxidant activityof *Bridelia retusa* plant. In future investigation is needed to isolate active constituents and to carry their *in vivo* antioxidant study of *Bridelia retusa* plant.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this research.

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