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Research Article

ANTIBACTERIAL ACTIVITY, FREE RADICAL SCAVENGING POTENTIAL, PHYTOCHEMICAL INVESTIGATION AND *IN-VIVO* TOXICITY STUDIES OF MEDICINAL PLANT *EMBELIA BASAAL* (R. & S.) A. DC.

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ABSTRACT

Objective: Present extensive study on medicinal plant *Embelia basaal* (R. & S.) A. DC. was undertaken for evaluation and quantification of broad spectrum antibacterial activity, free radical scavenging potential, phytochemical investigation, development of high performance thin layer chromatography (HPTLC) fingerprint profile and *in-vivo* safety of the plant.

Methods: Dried fruits of the plant were powdered and macerated in variety of solvents to obtain four extracts of different polarities. Antibacterial activity was evaluated using agar well diffusion assay. Most potent antibacterial extract of *E. basaal* was selected for further extensive studies. Minimum inhibitory concentration (MIC) values of the potent antibacterial extract were determined using broth macrodilution method as per Clinical Laboratory Standards Institute guidelines. Free radical scavenging activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl assay. Qualitative phytochemical tests, quantification of total phenolic contents and HPTLC fingerprint analysis were carried out for phytochemical investigation. The *in-vivo* safety of the plant was determined by conducting acute oral toxicity studies in mice as per Organization for Economic Co-operation and Development guidelines test no. 420.

Results: Ethanol extract of *E. basaal* was found potent bactericidal against selected pathogens and chosen for further extensive studies. The extract exhibited significant free radical scavenging activity (inhibitory concentration 50 value=50 µg/mL). Phytochemical investigation revealed presence of phenolic compounds, terpenoids, tannins and alkaloids in the test extract and it was found safe *in-vivo* at a dose level of 2000 mg/kg body weight of an animal.

Conclusion: *E. basaal* was found medicinally active in the present study. Its broad spectrum antibacterial activity, significant free radical scavenging potential and *in-vivo* safety will provide a platform for advance studies in the area.

Keywords: *Embelia basaal* (R. & S.) A. DC., Bactericidal activity, Free radical scavenging activity, Phytochemical investigation, High performance thin layer chromatography fingerprint, Acute oral toxicity.

INTRODUCTION

Embelia basaal (R. & S.) A. DC. (Syn. *Embelia tsjerium-cottam, Embelia robusta*) is a small tree or shrub from *Myrsinaceae* family, which grows up to 3 m high [1,2]. Its fruits and flowers are found throughout the year. The plant is distributed in the Western Ghats and central region of Maharashtra state [1]; and throughout in the greater part of India [3]. The plant is medicinally active and used in weak pulse rate [3], stem bark is used as a mucolytic and seed extract possesses antifungal activity against dermatophytes and related keratinophylic fungi [4]. Various preparations of the plant are effectively used in ascariasis [4]. The fruits of the *E. basaal* are said to have antispasmodic, carminative, anthelmintic, antibacterial, and anti-dysentery actions [3,4]. Fruits are also one of the ingredients of compound drug formulation "nilavarai curnam" [4].

Although *Embelia ribes*, an another medicinally important plant from same genus *Embelia*, is widely used and investigated in detail for its therapeutic properties; only a smaller amount of research work has been reported for the plant *E. basaal* which is used as substitute for *E. ribes* [2]. *E. basaal* is a large shrub and has a maximum fruit-bearing capacity [2]. It is widely distributed throughout the greater part of India; and as reported by Patwardhan *et al.,* (2010) [2], due to the non-availability of authentic *E. ribes*, market demand of *E. basaal* has been increased. *E. basaal* has been studied for its antimicrobial activity against some bacteria and salivary microflora [5-9]. Its free radical scavenging activity has also been reported [10]. But there are no reports available for *E. basaal* on the quantification of its antibacterial activity against various pathogens, qualitative phytochemical investigation for the detection of various phytochemical groups present, high performance thin layer chromatography (HPTLC) fingerprint profile and *in-vivo* safety. Hence, in order to generate scientific data, the present extensive study on *E. basaal* was undertaken for the quantification of its antibacterial activity by broth macrodilution method, phytochemical investigation, development of HPTLC fingerprint profile and to assess the *in-vivo* safety profile of the plant (acute oral toxicity studies). Estimation of antioxidant activity of the potent bactericidal extract of *E. basaal* and quantification of its total phenolic content; were also part of the present study.

METHODS

Collection and authentication of plant material

Dried fruits of *E. basaal* were procured from local herb dealer and authenticated with voucher specimen number F-179 from Agharkar Research Institute, Pune (India).

Extraction of *E. basaal* dried fruits

The dried fruits of *E. basaal* were powdered using mixer-grinder and sieved through American Standards for Testing Materials mesh size 40. The powdered plant material was extracted in four organic solvents differing in their polarities; namely n-hexane, dichloromethane, ethyl acetate, and ethanol. Extraction was carried out by the method of "kinetic maceration" [11] at room temperature where the plant

material was kept in constant motion in contact with solvents. 1 g plant powder was added to 10 mL each of the respective organic solvents in stoppered test tubes. The stoppered test tubes were then kept on rotary test tube shaker for extraction at room temperature with 30 rotations per minute for 6 hrs. The extracts were collected and filtered to eliminate the particulate matter and the solvents were removed under reduced pressure. For preliminary screening of antibacterial activity; all the four dried extracts were dissolved in dimethyl sulfoxide to a final stock concentration of 5% w/v.

Procurement of pathogenic bacterial strains

Authentic, pathogenic strains of six bacteria having important clinical significances in human were procured from microbial type culture collection (MTCC), Institute of Microbial Technology, Chandigarh (India).

The Gram-positive bacteria tested for the study were:

1. *Staphylococcus aureus*, MTCC No. 96, the pathogenic strain
2. *Staphylococcus epidermidis*, MTCC No. 435, isolated from the skin lesion
3. *Streptococcus mutans*, MTCC No. 497, isolated from carious dentine.

The Gram-negative (*which are *P. aeruginosa*, *P. mirabilis* and *S. marcescens*) bacteria tested for the study were:

1. *Pseudomonas aeruginosa,* MTCC No.3542, the pathogenic strain isolated from human
2. *Proteus mirabilis,* MTCC No. 425, isolated from urine of the patient with kidney stones
3. *Serratia marcescens,* MTCC No. 97, pathogenic strain isolated from pond water.

All the cultures (except *S. mutans*) were maintained on nutrient agar. *S. mutans* was maintained on brain heart infusion agar as per MTCC recommendations.

Preliminary screening of antibacterial activity

Preliminary screening of antibacterial activity to select most potent antibacterial extract of *E. basaal* for further studies was performed by agar well diffusion assay. Mueller-Hinton (MH) broth and MH agar (both manufactured by Himedia) were used for the assay.

All the bacterial cultures, which were freshly grown overnight; were adjusted to match the turbidity of 0.5 McFarland standard (approx. matching to 1.5×10^8 CFU/mL) using sterile MH broth. 1 mL each of the adjusted inoculum was mixed with the sterile molten (but cooled) MH agar and poured into sterile petri plates (Borosil). After solidification of the seeded agar in petri plates, wells of 8 mm diameter were punched through it using sterile cork borer. A volume of 50 µl of all the four test extracts of *E. basaal* were added to the wells bored and plates were incubated at 37°C for 24 hrs (except for *S. marcescens,* which was incubated at 30°C as per MTCC recommendations). After the incubation period, plates were observed for the clear zones of inhibition for the antibacterial activity of the test plant extracts. Zones of inhibition around the wells were measured in millimeters. For Gram-positive bacteria 0.02% v/v Clindamycin (Cipla Ltd., India) and for Gram-negative bacteria 0.01% v/v gentamicin (Aquafine Injecta Pvt. Ltd., Pune, India) were used as a positive control for antibacterial activity as per Clinical Laboratory Standards Institute (CLSI) guidelines [12]. This experiment was performed 3 times on three different days. Results are mentioned as mean of the three experiments with standard deviation values.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

For the quantification of antibacterial activity, MIC and MBC determination analysis was performed with the most potent antibacterial ethanol extract of *E. basaal*, shortlisted in the preliminary screening study. MIC and MBC values were determined, using broth macrodilution assay as per CLSI guidelines [13].

Phytochemical investigation

Qualitative phytochemical analysis was performed by the methods of Jones and Kinghorn, (2006) [14] and Sasidharan *et al*., (2011) [15] for the detection of various classes of phytochemicals present in the ethanol extract of *E. basaal.*

Determination of total phenolic content

Total phenolic content of ethanol extract of *E. basaal* was carried out using Folin–Ciocalteau reagent. To 1 mL of test sample equal volume of Folin–Ciocalteau reagent was added along with 2 mL of 10% w/v sodium carbonate solution. Mixture was vortexed and allowed to stand for 30 minutes after which the absorbance was measured at 730 nm using a spectrophotometer (JASCO V-630). Gallic acid was used as a standard. Total phenolic content of test extract was determined from the equation (y=0.045x+0.006) obtained by linear regression analysis of calibration curve (Fig. 1) of standard gallic acid (5-25 µg/mL) and was expressed as gallic acid equivalents (mg) per gram of the dried test extract. The assay was carried out in triplicates.

Free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method

Free radical scavenging activity of ethanol extract of *E. basaal* was evaluated by DPPH assay. DPPH reagent was prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol to obtain 0.1 mM concentration. Ascorbic acid dissolved in methanol was used a standard for the assay at concentration range of 10-34 µg/mL. Dried ethanol extract of *E. basaal* dissolved in methanol was used a test solution at a concentration range of 20-120 µg/ml. 3 mL each of the test extract solution was mixed with the 1 mL of the prepared DPPH reagent and allowed to react in dark for 45 minutes at room temperature. Later, the absorbance of all the test mixtures was measured by spectrophotometer (JASCO V-630) at 517 nm. Methanol was used as a blank and methanol added with DPPH reagent was used a control for the assay. Absorbance values of all the test samples and standards were recorded in triplicates. The free radical scavenging activity was calculated by following formula [16]:

$$\%\ \text{DPPH reduction} = \frac{(\text{Absorbance of control} - \text{absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

The inhibitory concentration 50 (IC_{50}) values was calculated using linear regression analysis for the test extract and compared with the IC_{50} value of ascorbic acid standard used.

HPTLC fingerprint profile

HPTLC fingerprint profile was developed for *E. basaal*. Pre-coated silica gel $60F_{254}$ TLC plates (Merck) were used for the analysis. 2 µl of bioactive ethanol extract (50 µg/µl) of *E. basaal* was applied on a TLC plate with 8 mm band length using CAMAG LINOMAT Applicator-V. Chromatographic separation of the extract was carried out using toluene:ethyl acetate:acetic acid (5:4:1 v/v/v) as a mobile phase, in

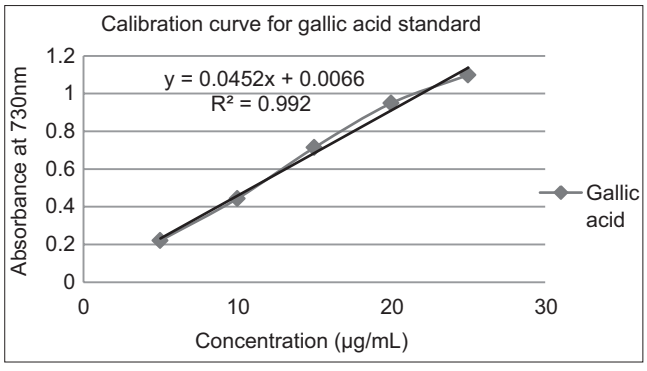

Fig. 1: Calibration curve obtained for gallic acid standard

a CAMAG twin trough chamber pre-saturated with mobile phase for 10 minutes. The TLC plate was run with mobile phase at a distance of 8 cm and densitometrically scanned using CAMAG Scanner-3 at wavelengths of 254 nm (absorption mode), 366 nm (fluorescence mode). The TLC plate was then derivatized using anisaldehyde-sulphuric acid reagent and densitometrically scanned at 540 nm (absorption mode).

Acute oral toxicity study

Acute oral toxicity study was carried out as per Organisation for Economic Co-operation and Development guidelines test no. 420, to assess the safety of the bioactive ethanol extract of *E. basaal*. Study was carried out at Animal Testing Centre, Ramnarain Ruia College, Mumbai (India); after an approval (KB-130620-01) from Institutional Animal Ethics Committee. Albino Swiss female mice were used for the study. Fixed dose method with dose concentration of 2000 mg/kg bodyweight of the animal, was followed. Parameters observed were cage side observations, daily food and water intake, daily body weight and mortality.

RESULTS

Kinetic maceration of *E. basaal* at room temperature using four different solvents resulted in different percent extraction yield for each solvent. The percent yield was in decreasing order from ethyl acetate (11.87%) > ethanol (11.49%) > dichloromethane (6.56%) > n-hexane (5.47%) as shown in Table 1. Overall the yield was more for mid-polar and polar solvents namely ethyl acetate and ethanol.

Results for the preliminary screening for antibacterial activity are mentioned in Table 1. The results are expressed as means of three different experiments performed with standard deviations. Ethanol extract of *E. basaal* exhibited potent broad-spectrum antibacterial activity against all the selected strains of pathogenic bacteria by forming zones of inhibition which were comparatively larger in diameters than zone diameters produced by other extracts. Hence, ethanol extract of *E. basaal* was selected for further extensive studies. Inhibition zone diameters of *E. basaal* ethanol extract ranged between 11.7 and 12.7 mm for the tested bacterial pathogens, and the largest inhibition zone diameter of 12.7 mm was observed against *S. mutans.* The zone diameters obtained for *E. basaal* ethanol extract were comparable with the positive standards (synthetic antibiotics) used for the study.

Determination of MIC and MBC of *E. basaal* ethanol extract was performed for the quantification of antibacterial activity. The results are given in Table 2. The MIC values ranged between 0.4 and 0.8%, whereas MBC values ranged between 0.8 and 1.6%. Lowest MIC of 0.4% was recorded for the test extract against *P. mirabilis*. A sample is said to be bactericidal in nature when the ratio of MBC/MIC ≤4 and bacteriostatic when this ratio is >4 [17]. The selected potent ethanol extract of *E. basaal* was proved to be bactericidal in nature as the MBC/MIC ratio obtained for all the pathogens was <4 (except for *S. aureus*, for which MBC was out of concentration range selected).

MBC/MIC≤4: Bactericidal activity [17].
MBC/MIC>4: Bacteriostatic activity [17].

Preliminary phytochemical screening was performed to detect the presence of various groups of phytochemicals present in the *E. basaal* ethanol extract which may be responsible for the bioactivities of the plant. The results are given in Table 3, revealed the presence of terpenoids, phenolic compounds, tannins and alkaloids in the test extract.

Total phenolic content in the test extract was quantified by Folin–Ciocalteau colorimetric assay in which gallic acid was used as a standard. The total phenolic content was found to be 5.8 mg/g of dried extract (in terms of gallic acid equivalents) (Table 4). Fig. 1 shows the calibration curve and linear regression analysis obtained for gallic acid standard used.

The free radical scavenging activity of the *E. basaal* ethanol extract was determined by DPPH assay for which the ascorbic acid was used as a standard. Table 5 shows the concentrations and % DPPH inhibition values of the test extract and the ascorbic acid standard. The IC_{50} value calculated for the test extract was 50.0 µg/mL; whereas for the ascorbic acid standard it was calculated as 17.9 µg/mL.

HPTLC fingerprint profile was developed for the *E. basaal* ethanol extract. The compiled peak table of the three wavelengths selected for densitometric scanning is given in Table 6 and the developed HPTLC plates are shown in Fig. 2. Three major peaks were observed for each of the three wavelengths scanned. At 254 nm (Fig. 3) major peaks were

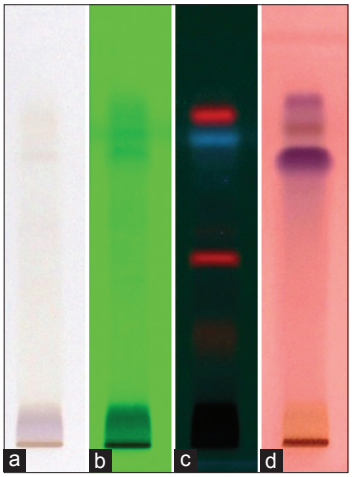

Fig. 2: Developed high performance thin layer chromatography plates for ethanol extract of *Embelia basaal*, (a) Under normal light, (b) under 254 nm, (c) under 366 nm, (d) under 540 nm after derivatization

Table 1: Results for preliminary screening of antibacterial activity by agar diffusion assay of four *E. basaal* extracts with their percent extraction yield

S. no.	Pathogenic bacteria	Diameter of zone of inhibition±SD of four *E. basaal* extracts with their percent extraction yield				DMSO (vehicle)	0.02% Clidamycin	0.01% Gentamicin
		Hex (5.47%)	DCM (6.56%)	EtOAc (11.87%)	EtOH (11.49%)			
1	*S. aureus* (MTCC 96)	9.3±0.58	9.7±0.58	10±1	11.7±0.58	-	11.7±0.58	Not tested
2	*S. epidermidis* (MTCC 435)	9±0	9.7±0.58	9.7±0.58	12.3±0.58	-	11.3±0.58	Not tested
3	*S. mutans* (MTCC 497)	10.3±0.58	10.7±0.58	11.3±0.58	12.7±0.58	-	15.7±0.58	Not tested
4	*P. aeruginosa* (MTCC 3542)	9±0	10.3±0.58	10.7±0.58	11.7±0.58	-	Not tested	12.3±0.58
5	*P. mirabilis* (MTCC 425)	9±0	9.3±0.58	11±0	11.7±0.58	-	Not tested	12.7±0.58
6	*S. marcescens* (MTCC 97)	9±0	9±0	9.7±0.58	11.3±0.58	-	Not tested	13.7±0.58

SD: Standard deviation, *S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, P. aeruginosa: Pseudomonas aeruginosa, P. mirabilis: Proteus mirabilis, S. marcescens: Serratia marcescens*, Hex: n-hexane, EtOAc: Ethyl acetate, DMSO: Dimethyl sulfoxide, DCM: Dichloromethane, EtOH: Ethanol, - : No zone of inhibition was observed, *E. basaal: Embelia basaal*, MTCC: Microbial type culture collection

Table 2: Results for the determination of MIC and MBC of ethanol extract of *E. basaal* as per CLSI guidelines

S. no.	Pathogenic bacteria	Ethanol extract of *E. basaal* (%)		MBC/MIC ratio
		MIC	MBC	
1	*S. aureus* (MTCC 96)	0.8	Above1.6	-
2	*S. epidermidis* (MTCC 435)	0.8	0.8	1
3	*S. mutans* (MTCC 497)	0.8	1.6	2
4	*P. aeruginosa* (MTCC 3542)	0.8	1.6	2
5	*P. mirabilis* (MTCC 425)	0.4	0.8	2
6	*S. marcescens* (MTCC 97)	0.8	1.6	2

Concentration range for the tested extract was 1.6-0.05% w/v. - : Not calculated. MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, *S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, P. aeruginosa: Pseudomonas aeruginosa, P. mirabilis: Proteus mirabilis, S. marcescens: Serratia marcescens*, MTCC: Microbial type culture collection, *E. basaal: Embelia basaal*

Table 3: Results for preliminary phytochemical screening of ethanol extract of *E. basaal*

S. no.	Phytochemical groups	Test	Reference	Results
1	Sterols	Liebermann-Burchard test	Jones and Kinghorn, 2006 [14]	-
2	Terpenoids	Salkowski test	Sasidharan *et al.*, 2011 [15]	+
3	Volatile oils	NaOH and H_2SO_4 test	Sasidharan *et al.*, 2011 [15]	-
4	Phenolic compounds	Ferric chloride test	Jones and Kinghorn, 2006 [14]	+
5	Flavonoids	Shinoda test	Jones and Kinghorn, 2006 [14]	-
6	Tannins	Gelatine-salt test	Jones and Kinghorn, 2006 [14]	+
7a.	Alkaloids	Mayer's reagent test	Jones and Kinghorn, 2006 [14]	+
7b.		Dragendorff's reagent test	Jones and Kinghorn, 2006 [14]	+
8	Saponins	Foam test	Jones and Kinghorn, 2006 [14]	-

+: Presence of phytochemical group, -: Absence of phytochemical group, *E. basaal: Embelia basaal*

Table 4: The total phenolic content of ethanol extract of *E. basaal*

Test extract	Total phenolic content
Ethanol extract of *E. basaal*	5.8 mg/g of dried extract (gallic acid equivalents)

E. basaal: Embelia basaal

observed at R_f 0.70, 0.75 and 0.79. At 366 nm (Fig. 4) major peaks were observed at R_f 0.43, 0.73 and 0.79 whereas at 540 nm (after derivatization) major peaks were observed at R_f 0.69, 0.76 and 0.82 (Fig. 5).

Acute oral toxicity study of *E. basaal* ethanol extract demonstrated no signs of toxicity at a dose level of 2000 mg/kg body weight of Albino Wistar mice. From the observations recorded for behavioural changes, body weight changes, cage side observations, clinical observations and mortality; the test extract was found safe in mice.

DISCUSSION

Comparatively higher extraction yields obtained for *E. basaal* dried fruit powder in the mid-polar and polar solvents suggests that the plant material contains marked amount of mid-polar and polar phytoconstituents, which could have been extracted in solvents like ethyl acetate and ethanol. This is based on the principle of "like-dissolves-like," since generally; lipophilic components in plants such as alkanes, fatty acids, sterols, and some terpenoids are extracted in nonpolar solvents; whereas more polar components such as glycosides, polyphennols, and tannins are extracted in more polar solvents [18]. Qualitative phytochemical investigation of *E. basaal* also supports this fact; as the presence of polar compounds such as phenolic acids, tannins and alkaloids was detected in the polar ethanol extract under study.

Table 5: Percent DPPH inhibition recorded for ethanol extract of *E. basaal* and for ascorbic acid standard

S. no.	% DPPH inhibition for ethanol extract of *E. basaal*		% DPPH inhibition for ascorbic acid	
	Concentration (µg/ml)	Mean	Concentration (µg/ml)	Mean
1	20	29.13	10	26.48
2	40	37.69	14	38.85
3	60	59.99	18	43.13
4	80	73.26	22	66.12
5	100	91.17	26	79.26
6	120	96.41	30	86.48
7	-	-	34	98.18
	IC_{50} value=50.0 µg/ml		IC_{50} value=17.9 µg/ml	

DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC_{50}: Inhibitory concentration 50, *E. basaal: Embelia basaal*

Table 6: Peak table for HPTLC analysis of *E. basaal* at 254 nm, 366 nm and 540 nm

Wavelength selected for densitometrica scanning	Peak number	Maximum R_f	Peak area	% peak area
254 nm (absorption mode)	1	0.38	1545.4	4.87
	2	0.43	942.8	2.97
	3	0.53	2381.9	7.51
	4	0.62	2173.4	6.85
	5	0.70	8200.0	25.86
	6	0.75	9561.8	30.16
	7	0.79	6900.5	21.76
366 nm (florescence mode)	1	0.11	681.6	3.11
	2	0.23	2065.1	9.42
	3	0.24	1798.1	8.20
	4	0.43	5000.0	22.80
	5	0.49	568.9	2.59
	6	0.63	552.5	2.52
	7	0.73	5615.5	25.61
	8	0.79	5647.1	25.75
540 nm (asorption mode, after derivatization)	1	0.33	1565.7	2.37
	2	0.69	43145.3	65.37
	3	0.76	10062.4	15.25
	4	0.82	11229.4	17.01

HPTLC: High performance thin layer chromatography, *E. basaal: Embelia basaal*

The total phenolic content of ethanolic test extract was found to be 5.8 mg/g of dried extract (gallic acid equivalents) whereas; Kamble *et al.*, (2011) [10] have reported total phenolic content of ethanol extract of *E. basaal* as 65.6 mg/g of dried extract in terms of pyrrocatechol equivalents. The differences in the results obtained and reported data may be due to different extraction procedures, protocols followed for estimation and standards used to express total phenolic contents by investigators. The IC_{50} value of the ethanolic test extract for DPPH assay was found to be 50.0 µg/mL in present study, in comparison with the IC_{50} value of ascorbic acid standard used, which was 17.9 µg/mL. Whereas the IC_{50} values of ethanol extract of *E. basaal* and the ascorbic acid standard were reported as 9.87 µg/mL and 3.028 µg/mL respectively by Kamble *et al.*, (2011) [10]. Again

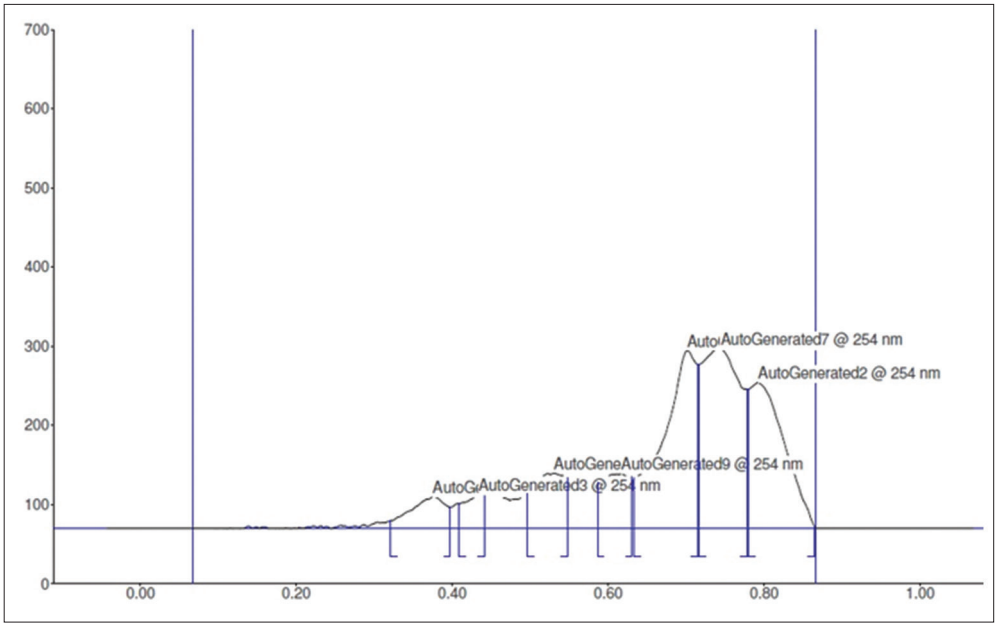

Fig. 3: High performance thin layer chromatography chromatogram of ethanol extract of *Embelia basaal* at 254 nm

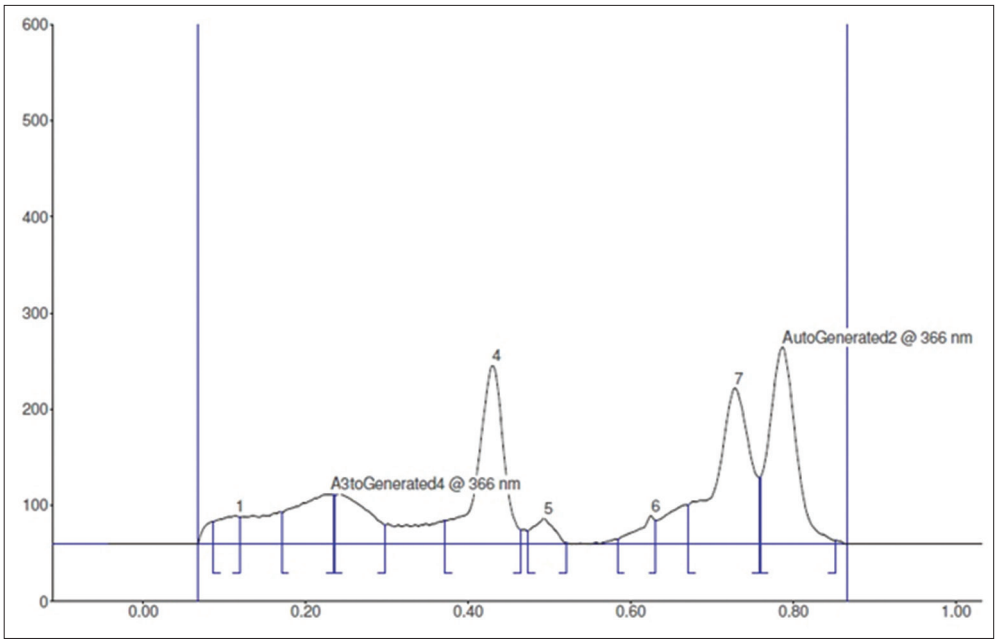

Fig. 4: High performance thin layer chromatography chromatogram of ethanol extract of *Embelia basaal* at 366 nm

the differences may be due to the different extraction procedures and protocols followed for the assay. The presence of phenolic compounds could be responsible for the significant free radical scavenging activity of the crude test extract since phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes are said to be responsible for radical-scavenging activity of plant products [19]. Also many studies in this area have reported that the phenolic and flavonoid contents in the plants can be correlated to their antioxidant/free radical scavenging activity [20]. Overall, the ethanolic extract of *E. basaal* exhibited significant antioxidant potential which can be helpful in reducing risks of various diseases related to oxidative stress [20].

E. basaal exhibited broad spectrum antibacterial activity as earlier reported by Kamble *et al.*, (2011) [5]. The ethanol extract of *E. basaal* was found most potent in preliminary screening studies for antibacterial activity and demonstrated bactericidal potential (MBC/MIC ratio <4) against six clinically significant bacterial pathogens which are responsible for causing array of infections in human such as skin and soft tissue infections, urinary and respiratory tract infections, various nosocomial infections, dental caries, eye infections etc. Presence of bioactive phytoconstituents such as phenolic acids, tannins, alkaloids and terpenoids in ethanol extract of *E. basaal* may be responsible for its antibacterial activity.

HPTLC fingerprint is the simplest way for identification of plant material. It can help in rapid detection of classes of phytoconstituents and it can also be coupled with the techniques like bioautography, for the bioactivity guided isolation of bioactive compounds such as antimicrobial and antioxidant compounds [21,22]. Under 254 nm (short ultraviolet [UV]) few dark bands were observed against florescent green background of HPTLC plate; which indicated presence of compounds having conjugated double bonds [21]. Under 366 nm

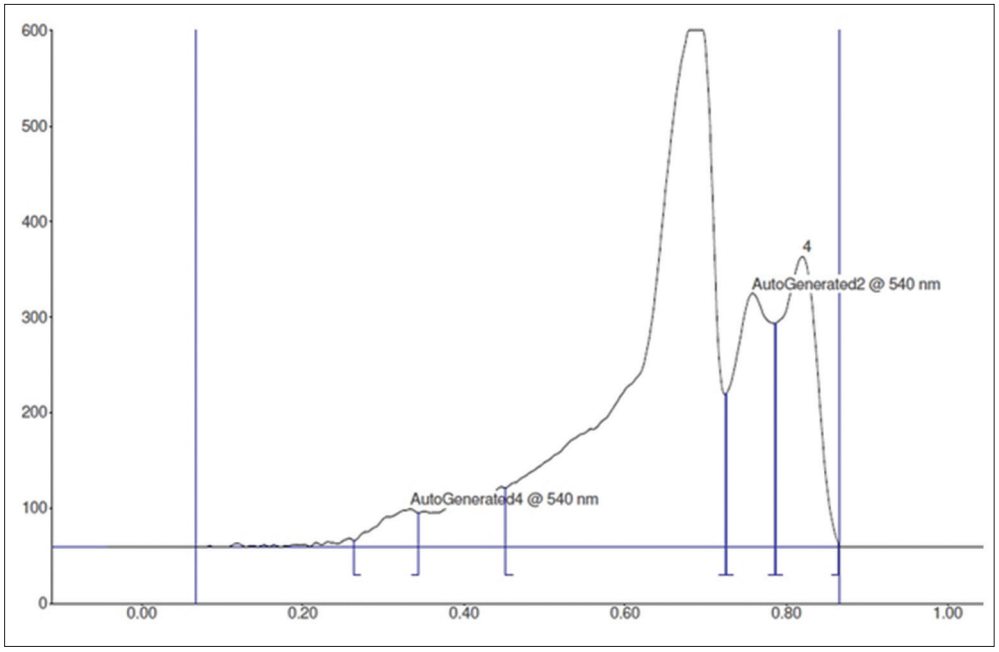

Fig. 5: High performance thin layer chromatography chromatogram of ethanol extract of *Embelia basaal* at 540 nm after derivatization

(long UV), HPTLC fingerprint of test extract illustrated the presence of florescent bands which indicated the possible presence of alkaloids, anthracene derivatives, coumarins, flavonoids (phenolic compounds) etc. in the test extract [21]. Treatment of developed HPTLC plate with anisaldehyde - sulfuric acid reagent produced various colored bands. The reagent is generally used for detecting presence of various phytoconstituents such as essential oil components, steroids, glycosides, saponins and phenols [23]. Thus, the HPTLC fingerprint profile was developed for *E. basaal* and the results of the analysis were found matching with the results of preliminary phytochemical screening which revealed presence of terpenoids, phenolic acids, tannins and alkaloids in the test extract.

Acute oral toxicity studies demonstrated the safety of the extract at a dose level of 2000 mg/kg bodyweight of the Albino Wistar mice and justified the use of the plant in traditional medicinal preparations as well as provided *in-vivo* scientific data for the safety index of the plant for future research work.

CONCLUSION

E. basaal which is used as a substitute for *E. ribes,* was found medicinally active in the present study. The broad spectrum antibacterial activity, significant free radical scavenging activity, phytochemical investigations, HPTLC fingerprint profile and *in-vivo* safety of the plant, were studied extensively. Significant data generated in present study will provide a platform for advance research in the area. Further isolation, purification and identification of antioxidant compounds along with fractionation, bioassay guided isolation and identification of antibacterial compounds from *E. basaal* may provide some important leads in the area.

Currently at our lab, the work is in progress for bioassay-guided isolation and identification of antibacterial and antioxidant constituents of this plant.

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