Research Article

NOVEL ANTIPROPIONIBACTERIUM ACTIVITY OF EMBELIN AND CHEBULAGIC ACID ON SCREENING OF INDIAN MEDICINAL PLANTS

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ABSTRACT

Modern antibiotics against acne vulgaris has led to resistance among *Propionibacterium acnes*. This may be overcome with the use of ancient Indian medicines documented against skin diseases. Methanolic extracts of 21 medicinal plants were screened for antipropionibacterium activity to provide a scientific basis for indigenous cures derived from traditional knowledge. Seven medicinal plants were found to inhibit the growth of *P. acnes*. Of these *Embelia ribes*, *Salmalia malabarica* and *Terminalia chebula* showed strong inhibitory effects. The novel antipropionibacterium activity of embelin isolated from *Embelia ribes* and chebulagic acid isolated from *Terminalia chebula* which strongly inhibited the growth of *P. acnes* with minimum inhibitory concentration of 12.5 µg/ml has been reported.

Keywords: acne, embelin, antibacterial activity, minimum bactericidal concentration.

INTRODUCTION

Acne vulgaris is a common skin disorder of the sebaceous follicles involving various pathogenic factors such as seborrhea¹, ductal hypercornification of keratinocytes² stimulation of sebaceous gland secretion causing hormone imbalance, inflammation³ and microbial colonization with *Propionibacterium acnes*⁴. *P. acnes* produce an exoenzyme lipase that hydrolyses sebaceous triglycerides, releasing free fatty acids causing irritation of the follicular wall and the surrounding dermis^{5,6}. Several studies have been conducted globally to prove the efficacy of phytochemicals. India is a rich emporium of medicinal plants, mainly used in preventive and curative medicine⁷. This heritage based on ayurvedic and homeopathic systems of medicine, is predominantly plant based and materia medica has already been compiled in ancient Indian texts such as Rigveda, Sushruta Samhita and Charaka Samhita. Acne in severe form requires medical attention, but during early stages and mild forms can be treated effectively with natural products. Furthermore, these products help in removing blemishes. The increasing failures of chemotherapeutics and antibiotic resistance exhibited by pathogens have led to the screening of several medicinal plants for their potential antimicrobial activity^{8,9}. Cosmetic formulations containing such plant extracts can protect the skin against harmful exogenous or endogenous agents and cure many skin conditions like acne, psoriasis, eczema and dandruff¹⁰.

Here, we investigated the efficacy of twenty one Indian medicinal plants as potential drug candidates against *Propionibacterium acnes*. Plants such as *Salmalia malabarica*, *Symplocos racemosa*, *Vitex negundo*, *Terminalia chebula*, *Terminalia bellerica*, *Picrorhiza kurroa* and *Embelia ribes* that have been previously cited in books like Charaksamhita¹¹ and

Dravyagunvidyan¹² were selected for their astringent, antibacterial, anti-inflammatory, antitumor and several other ethnobotanical properties as listed in Table 1¹³⁻²⁴. However, their activity against *P. acnes* has not been investigated so far. Extracts from above plants were found to have significant antiacne activity. This investigation showed that embelin isolated from the chloroform extract of *Embelia ribes* and chebulagic acid isolated from *Terminalia chebula* exhibited the highest antipropionibacterium activity. Earlier reports on embelin revealed antifertility, analgesic, anti-inflammatory, antioxidant and antitumour properties^{25, 26}. Chebulagic acid is a potent α glucosidase inhibition, blocks T lymphocyte cytotoxicity and moderately cytotoxic against human tumor cell lines²⁷⁻²⁹.

MATERIALS AND METHODS

Plant collection and authentication

The plants used in this study were collected from the suburbs of Mumbai, dried and powdered. These plant specimens were identified and authenticated by Dr. Vinayak Naik and specimens were deposited in the Herbarium of Zandu Pharmaceuticals Works Ltd, Mumbai, India (Table 1).

Reagents and chemicals

Brain heart infusion agar, brain heart infusion broth, tryptone soya agar, saboraud agar, blood agar and nutrient agar (HiMedia Pvt. Ltd. India) were used for culturing the test organisms. Petroleum ether, diethyl ether, chloroform, methanol and benzoyl peroxide were obtained from Sigma-Aldrich (St. Louis, USA). Benzoyl peroxide and erythromycin esteolate (Pfizer Ltd. India) were used as control.

Preliminary phytochemical screening and successive solvent extraction

The air dried powdered plant material of all the plants except *E. ribes* were extracted successively with solvents such as petroleum ether, chloroform and methanol (15 g powder in 150 ml of each solvent) using Soxhlet's apparatus. The seeds of *E. ribes* were extracted with diethyl ether, chloroform and methanol. Prior to extraction with each solvent, the powdered material was dried in an oven below 50°C. Finally, the residue was macerated with water for 24 h to obtain an aqueous extract and the yield was calculated. Alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins and amino acids were qualitatively examined. The major constituents, tannins, glycosides and alkaloids were isolated quantitatively^{30,31}. The chloroform extract of *E. ribes* was further extracted with cold petroleum ether. Extract was evaporated to dryness and residue extracted with chloroform. Embelin was crystallized from the above extract³² and analysed qualitatively using HPLC³³. Embelin crystals were further analysed by NMR, FT-IR and mass spectroscopy³⁴.

The crude methanolic extract of *T. chebula* was fractionated with *n*-hexane, chloroform and 1-butanol³⁵. The 1-butanol fraction of *T. chebula* was analyzed by HPTLC using silica gel 60 F-254 plates (E. Merck, Darmstadt, Germany). Quantitation of chebulagic acid, ellagic acid, and gallic acid was performed using ethyl formate: dichloromethane: acetic acid: formic acid (5:5:4:2) as mobile phase and that of tannic acid by using toluene: chloroform: methanol (4:4:1)³². The plate was dipped in ferric chloride reagent and heated at 100 °C for 3 min. For isolation of chebulagic acid, samples were streaked lengthwise on 10 × 20 cm plates. Bands were visualized under UV light at 254 nm and individually scraped.

Collection and preservation of the culture

The cultures of *P. acnes* *737 (MTCC 1951) and *Staphylococcus epidermidis* (MTCC 3615) were obtained from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India. Other test organisms used in this study such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231) and *Streptococcus pyogenes* (MTCC 442) were procured from Lilavati Hospital, Mumbai, India.

The anaerobic microorganism *P. acnes* was grown using brain heart infusion agar, blood agar and aerobic microorganisms in tryptone soya agar and *C. albicans* in saboraud agar. *P. acnes* strain was identified as catalase, indole, nitrate and gelatin hydrolysis positive³⁶.

Evaluation of antibacterial activity by cup plate diffusion method

For antibacterial testing, a stock solution of each plant extract (5 mg/ml) was prepared. *P. acnes* cell density was adjusted to approximately 3×10^8 CFU/ml for inoculation and the agar wells were filled with 150 µl of each of the desired plant extract and incubated at 37°C for 48 h under anaerobic conditions. The aerobic organisms were inoculated in tryptone soya agar and

fungal organisms in saboraud agar. The procedures were as mentioned above except that the plates were incubated at 37°C for 24 h under aerobic conditions. The size of cork borer was 6 mm in diameter. All cup plate diffusion tests were performed in triplicates and the antibacterial activity was expressed as the mean diameter of zone of inhibition (ZOI in mm). The positive controls such as erythromycin (150 µl of 1 mg/ml) and benzoyl peroxide (150 µl of 4 mg/ml) were tested by the same method. The antimicrobial activity of embelin and chebulagic acid (25 µl of 5 mg/ml) was evaluated by disc diffusion method^{37,38}.

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

The plant extracts were diluted appropriately in brain heart infusion broth supplemented with 0.03% sodium thioglycollate to reduce the redox potential for *P. acnes* and in tryptone soya broth for *S. epidermidis*. The standardised bacterial suspension of *P. acnes* and *S. epidermidis* were added to serial dilutions of each plant extract giving an initial count of approximately 10^6 CFU/ml of culture in triplicates. These were further incubated anaerobically at 37°C for 2 days for *P. acnes* and aerobically at 37°C for 1 day for *S. epidermidis*. Lowest concentration to inhibit the growth of microorganisms was recorded as MIC. To determine the MBC, a loopful of broth was collected from the tubes that did not show any growth and was inoculated on nutrient agar for *S. epidermidis* and blood agar for *P. acnes*. The concentration at which no visible growth was seen was noted as the MBC³⁹.

Statistical analysis

The statistical analysis was determined using GraphPad Instat® software. The results of all assays were expressed as mean ± S.D. The ZOI for each microorganism were analyzed through one way analysis of variance (ANOVA) using the Dunnett's multiple comparisons test. P-value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Herbal medicine is an outcome of therapeutic experiences accumulated through generations by physicians practicing indigenous systems of medicine. Currently, clindamycin, erythromycin and benzoyl peroxide are being used to treat acne. However these compounds induce drug resistance in *P. acnes*⁴⁰. Thus, there is continuous effort for screening of novel natural products with antimicrobial activity. In this study, methanolic extracts of several plants have been screened for their antimicrobial activity against *P. acnes*. Of the 21 Indian medicinal plant extracts screened, 7 showed significant activities (Table 2), 3 plants were used as positive controls and 11 plants failed to show any activity against *P. acnes*. Among these plant extracts maximum antibacterial activity against *P. acnes* was seen with chloroform extracts of *Embelia ribes* (ZOI: 45 mm) and methanolic extracts from *T. chebula*, *S. malabarica* (ZOI: 43 mm). *T. bellerica*, *V. negundo* and *P. kurroa* (ZOI: 35–37 mm) also showed good activity. The ZOI obtained with plant extracts was comparable with erythromycin (40 mm) and benzoyl peroxide (34 mm).

Table 1: Ethnomedicinal plant species investigated

Botanical name	Family	Local name	Plant parts used	Voucher specimen number	Ethnomedicinal uses
<i>Symplocos racemosa</i> Wight ex C.B. Clark	Symplocaceae	Lodhra	Stem bark	ZPS-1870	Astringent, treatment of bowel complaints and ulcers
<i>Salmalia malabarica</i> DC Schott. and Endl.	Bombacaceae	Shalmali	Thorn	ZPS-2013	Pulmonary tuberculosis, dysentery, skin disorders
<i>Picrorhiza kurroa</i> Royle ex benth	Scrophulariaceae	Kutaki	Roots	ZPS-0671	Hepatoprotective, skin diseases.
<i>Vitex negundo</i> Linn	Lamiaceae	Nirgundi	leaves	ZPS-1209	Bactericidal, antitumor, insecticidal, fumigant, skin disorders.
<i>Embelia ribes</i> Burm.f.	Myrsinaceae	Vavding	Seeds	ZPS-1638	Anthelmintic, skin diseases, tumors, ascites, wound healing, leprosy.
<i>Terminalia chebula</i> Retz	Combretaceae	Harada	Fruit	ZPS-1154	Hyperlipidemia, skin diseases, diarrhoea, vaginitis.
<i>Terminalia bellerica</i> Roxb	Combretaceae	Behada	Fruit	ZPS-0386	Hyperlipidemia, diarrhoea, respiratory tract infections, cough, sore throat.
<i>Azadirachta indica</i> A. Juss	Meliaceae	Neem	leaves	ZPS-1187	Acne, antiviral, antihelmintic, antifungal,
<i>Curcuma longa</i> Linn	Zingiberaceae	Haldi	rhizome	ZPS-0321	Insect stings, inflammation, internal injuries, pimples, as a skin tonic
<i>Berberis aristata</i> DC	Berberidaceae	Daru haldi	rhizome	ZPS-2876	Inflammation, ulcers, jaundice conjunctivitis
<i>Cyperus rotundus</i> Linn.	Cyperaceae	Mustak	tuber	ZPS-0078	Fever, dysmenorrhea,
<i>Ficus racemosa</i> Linn.	Moraceae	Umber	root	ZPS-1430	Skin diseases, diarrhea, menorrhagia
<i>Quisqualis indica</i> Linn.	Combretaceae	Madhumalti	flower	ZPS-1849	Antihelmintic, wound infections
<i>Tinospora cordifolia</i> Miers.	Menispermaceae	Guduchi	root	ZPS-0081	Antitubercular, hepatotoxic, antitubercular
<i>Cassia fistula</i> Linn.	Caesalpiniaceae	Golden shower	bark	ZPS-1253	Constipation
<i>Adhatoda vasica</i> Nees.	Acanthaceae	Adulsa	root	ZPS-0501	Bronchodilator, asthma
<i>Trichosanthes dioica</i> Roxb.	Cucurbitaceae	Patola	root	ZPS-2163	Skin diseases, ulcer
<i>Aegle marmelos</i> Linn.	Rutaceae	Bael	root	ZPS-0839	Antiviral, anthelmintic, anti-inflammatory
<i>Saussurea lappa</i> C. B. Clarke.	Compositae	Kuth	root	ZPS-1801	Astringent, ulcers Respiratory infections
<i>Cassia tora</i> Linn	Caesalpiniaceae	Senna	root	ZPS-7390	Eczema, psoriasis, antifungal
<i>Alstonia scholaris</i> Linn.	Apocynaceae	Saptaparna	seed	ZPS-0613	Diarrhoea, malaria

Table 2: Phytochemicals screening, percent yield and antimicrobial activity against *P. acnes* of the plants studied

Botanical name (Voucher code)	Phytochemicals identified ¹	Fraction extract ²	Yield (% w/w)	ZOI ³ (mean $\pm$ SD) in mm
<i>Symplocos racemosa</i> Wight ex C.B. Clark (ZPS-1870)	AL, TA, GL, CBH, TE	I	2.13	-
		II	2.83	-
		III	13.53	21 $\pm$ 0.9
		IV	3.61	-
<i>Salmalia malabarica</i> DC Schott. and Endl. (ZPS-2013)	AL, TA, SA, CBH, TE	I	0.75	-
		II	0.47	-
		III	13.81	43 $\pm$ 0.5
		IV	0.61	-
<i>Picrorhiza kurroa</i> Royle ex benth (ZPS-0671)	SA, TA, GL, CBH, TE	I	1.46	-
		II	1.05	-
		III	8.44	35 $\pm$ 0.5
		IV	2.41	-
<i>Vitex negundo</i> Linn (ZPS-1209)	SA, AL, TA, GL, AA, CBH, TE	I	4.61	21 $\pm$ 0.9
		II	0.56	-
		III	4.63	36 $\pm$ 0.5
		IV	3.3	-
<i>Embelia ribes</i> Burm.f. (ZPS-1638)	AL, TA, AA, CBH, QU, TE	V	2.13	38 $\pm$ 0.9
		II	5.32	45 $\pm$ 0.5
		III	0.46	22 $\pm$ 0.5
		IV	4.13	-
<i>Terminalia chebula</i> Retz (ZPS-1154)	SA, AL, TA, GL, AA, CBH, TE	I	4.83	31 $\pm$ 0.5
		II	5.47	25 $\pm$ 0.8
		III	39.17	43 $\pm$ 0.5

<i>Terminalia bellerica</i> Roxb (ZPS-0386)	SA, AL, TA, GL, CBH, TE	IV	6.17	-
		I	4.27	22±0.5
		II	4.33	31±0.8
		III	37.66	37±0.5
		IV	5.75	-
Erythromycin ⁴	NA	NA	NA	40±0.4
Benzoyl peroxide ⁴	NA	NA	NA	34±0.5
I, II, III, IV, V ⁵	NA	NA	NA	-

¹AL: alkaloids, AA: amino acids, G: glycosides, QU: quinones, TA: tannins, TE: terpenoids, SA: saponins. ²I: petroleum ether, II: chloroform, III: methanol, IV: water, V: diethyl ether. NA: Not applicable, ³Values are means of three replications, ⁴Positive control, ⁵Negative control, (-): no inhibition of growth at the concentrations tested, P value < 0.05

Table 3: Antimicrobial activity of phytochemicals against *P. acnes* using cup plate diffusion method

Botanic name	Phytochemicals ¹	Yield (% w/w)	Zone diam of inhibition (mean ± SD) in mm
<i>Salmaia malabarica</i>	I	0.4	-
	II	0	NT
	III	0.82	24±0.9
<i>Embelia ribes</i>	I	1.04	-
	II	0	NT
	III	0.89	-
<i>Terminalia chebula</i>	I	0.04	-
	II	2.9	25±0.5
	III	4.467	40±0.5
Negative control (methanol)	NA	NA	-

¹I: alkaloids, II: glycosides, III: tannins. (-): no inhibition of growth at the concentrations tested. NA: Not applicable, NT: Not tested

Table 4: Antimicrobial activity of methanolic extracts against microorganisms by cup plate diffusion method

Plant name	Zone diameter of inhibition ¹ (mean± SD) in mm						
	<i>P. aeruginosa</i>	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>C. albicans</i> *	<i>S. pyogenes</i>
<i>S. racemosa</i>	16±0.5	24±0.9	23±0.5	-	13±0.5	16±0.9	38±0.5
<i>S. malabarica</i>	25±0.5	16±0.9	21±0.9	-	16±0.5	16±0.5	22±0.5
<i>P. kurroa</i>	17±0.5	16±0.5	11±0.5	12±0.9	21±0.5	18±0.5	22±0.5
<i>V. negundo</i>	12±0.5	12±0.9	12±0.5	13±0.7	15±0.5	15±0.5	18±0.5
<i>E. ribes</i>	13±0.5	16±0.9	21±0.9	13±0.9	22±0.8	12±0.5	20±0.5
<i>T. chebula</i>	18±0.5	21±0.5	17±0.5	16±0.7	22±0.5	20±0.5	25±0.5
<i>T. bellerica</i>	18±0.5	18±0.5	17±0.9	14±0.9	21±0.5	20±0.5	23±0.5
Methanol ²	-	-	-	-	-	-	-
Ampicillin ³ (10µg/ml)	25±0.5	22±0.3	24±0.2	23±0.1	28±0.2	15±0.2	19±0.5

¹Values are means of three replications, ²Negative control, ³Positive control, (-): no inhibition of growth at the concentrations tested.

* *C. albicans*- dimorphic fungus

Table 5: MBC values of plant extracts against *P. acnes* and *S. epidermidis* by broth dilution method

	Plants	MBC µg/ml		MIC µg/ml	
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>P. acnes</i>	<i>S. epidermidis</i>
A	<i>S. racemosa</i>	300	1800	100	900
B	<i>S. malabarica</i>	200	1700	75	800
C	<i>P. kurroa</i>	150	1000	50	700
D	<i>V. negundo</i>	400	1900	150	900
E	<i>E. ribes</i>	100	1000	50	400
F	<i>T. chebula</i>	100	1000	50	400
G	<i>T. bellerica</i>	200	1300	75	600
H	Erythromycin ¹	80	900	60	400
I	Benzoyl peroxide ¹	600	1200	600	600
J	Methanol ²	-	-		

(-): no inhibition of growth at the concentrations tested, ¹Positive control, ²Negativecontrol

Figure 1: ¹H NMR of embelin isolated from chloroform extract of *Embelia ribes*

Figure 2: Mass spectra of embelin

Purity Table:

DAD: Signal A,

Results				
Retention Time	Area	Area %	Height	Height %
2.947	9653	0.25	1468	0.28
3.273	15217	0.39	1787	0.34
3.853	3800362	98.11	515882	98.53
5.540	48483	1.25	4452	0.85
Totals	3873715	100.00	523589	100.00

Figure 3: HPLC of embelin isolated from chloroform extract

Figure 4: Images at 254 nm: lanes 1, 6, 7: methanolic extract of *T. chebula* (10 µl), lane 2: chebulagic acid (2 µl), lane 3: Chebulagic acid isolated from 1 butanol fraction (10 µl), lane 8: gallic acid (2 µl), lane 4, 5: ellagic acid (5 µl and 2 µl, respectively).

Figure 5: HPTLC images of tannic acid and gallic acid at 255 nm, 366 nm, 566 nm: Track 1, 2: methanolic extract of *S. malabarica* (5 µl and 10 µl of 5 mg/ml), Track 3, 4: tannic acid (5 µl, 10 µl) Track 6: methanolic extract of *T. chebula* (10 µl)

Since the maximum zone of inhibition was obtained with extract of *E. ribes*, *T. chebula* and *S. malabarica*, these extracts were used for further analysis. The chloroform extract from *E. ribes* was found to contain 33% of embelin. The chloroform residue was further extracted with petroleum ether and embelin was recrystallised with chloroform to obtain an orange crystalline compound. The λ_{max} for embelin was at 298 nm and m.p. was 144°C. On further analysis by ^1H NMR (500 MHz, DMSO-d_6) the compound was identified as 2,5-dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione (Figure 1). The ^1H NMR spectra showed δ -values at 5.779 (s) ppm representing one aromatic ring proton, 7.282 (s) ppm for OH proton, 2.443-2.496 (t) ppm for –CH - protons in the ring, 1.145-1.336 (m) ppm for $(\text{CH}_2)_{10}$ and 0.774-0.787 ppm for methyl group. Purity of isolated embelin was 98% as determined by HPLC (Figure 3) and further confirmed by mass spectroscopy with the molecular ion peak at m/z 293.2 (Figure 2) and FT-IR: (cm^{-1}) 3310, 2920, 2848, 1616, 1329, 1184. The spectral data was consistent with previously reported results^{34,41}.

The ZOI of embelin extracted from the chloroform extract of *E. ribes* was found to be 28 mm against *P. acnes* as determined by disc diffusion method. The concentration of embelin taken in this experiment for antiacne activity (125 µg) was almost 6 times lesser than the concentration of other plant extracts (750 µg). These experiments therefore indicated that the isolated embelin was a potent inhibitor for *P. acnes*.

The constituents isolated quantitatively in this study were alkaloids, glycosides, and tannins from *S. malabarica*, *E. ribes* and *T. chebula*. Table 3 represent the antipropionibacterium activities of the individual phytochemicals. It was seen that the zone of inhibition for tannins isolated from *T. chebula* (40mm) were highest against *P. acnes*. Tannins from *S. malabarica* and

glycosides from *T. chebula* exhibited moderate activity (21-25 mm) against *P. acnes* whereas alkaloids did not show any antimicrobial activity. The major constituents of tannins identified from *S. malabarica* were tannic acid, gallic acid and sugars. The major constituents identified and isolated from *T. chebula* using HPTLC were chebulagic acid, gallic acid and ellagic acid (supplementary data).

Plant extracts used as positive standards against *P. acnes* were from *Azadirachta indica* A. Juss (Meliaceae) leaves, *Curcuma longa* Linn (Zingiberaceae) rhizome, *Berberis aristata* DC (Berberidaceae) rhizome because of their well established antibacterial properties against *P. acnes*⁴². The ZOI obtained for methanolic extract of plants used as positive control was *A. indica* (42 mm), *C. longa* (40 mm) and *B. aristata* (41 mm) comparable with that of *E. ribes*, *T. chebula* and *T. bellerica*.

Methanolic extracts of the plants such as *Cyperus rotundus* Linn., *Ficus racemosa* Linn., *Quisqualis indica* Linn., *Tinospora cordifolia* Miers., *Cassia fistula* Linn., *Adhatoda vasica* Nees., *Trichosanthes dioica* Roxb., *Aegle marmelos* Linn., *Saussurea lappa* C. B. Clarke., *Cassia tora* Linn. and *Alstonia scholaris* Linn. failed to show significant antibacterial activity (ZOI < 13 mm) against *P. acnes*.

Since methanolic extract of most plants are found to be effective against *P. acnes*, their antibacterial activity against organisms such as *P. aeruginosa*, *B. subtilis*, *E. coli*, *S. aureus*, *S. epidermidis*, and *S. pyogenes* have been investigated as shown in Table 4. The antifungal activity against *C. albicans* was also investigated. Recently, Panda et al. (2009)¹³ have reported antibacterial activity (ZOI: 11-13.3) of *V. negundo* leaf extract at a concentration of 2500 µg/ml. Here, a similar inhibitory activity at much lower concentration (500 µg/ml, ZOI: 12-18 mm) has

been reported for *V. negundo*. Our findings are similar to recently reported antibacterial activities of *S. racemosa* and *P. kurroa*^{21,43}. In this study, extracts of *T. chebula* and *T. bellerica* showed broad spectrum antibacterial activity against all Gram positive and Gram negative organisms and maximum activity was shown against *P. acnes* and *S. epidermidis*. The ZOI obtained for extracts of *T. chebula* and *T. bellerica* (ZOI: 18-23 mm) in this study were much larger at lower concentration (750 µg) as compared to that obtained by earlier antibacterial studies^{44,21,45}. The reason for this variation may be due to difference in geo-climatic and environmental conditions, strain of organism, method of processing, extraction of plant material and assessment of antibacterial activity⁴⁶.

It has been previously reported that embelin from *Oxalis erythrorhiza* inhibited dematophytes (MIC: 50 µg/ml), *Microsporum gypseum* (MIC: 100 µg/ml), methicillin resistant *S. aureus* (MIC: 62 µg/ml) and sensitive *S. aureus* (MIC: 250 µg/ml)^{41,47}. Here, the MBC and MIC values for different plant extracts against *P. acnes* were in the range (100-400 µg/ml and 50-150 µg/ml respectively) as shown in Table 4. Methanolic extract of *T. chebula* and chloroform extract of *E. ribes* showed the lowest MIC value (50 µg/ml) and methanolic extract of *V. negundo* had highest MIC value (150 µg/ml). The MBC and MIC value of embelin and chebulagic acid (50 µg/ml and 12.5 µg/ml respectively) was found to be lower than erythromycin and benzoyl peroxide whereas ellagic acid, tannic acid and gallic acid showed MIC of 800 µg/ml, 1000 µg/ml and 1800 µg/ml respectively. The methanol control (1% v/v) showed no antimicrobial activity. The MBC and MIC value against *S. epidermidis* were found to be higher as compared to *P. acnes* as shown in Table 5.

On screening several Indian medicinal plants, this study showed that extracts of *E. ribes*, *T. chebula*, and *S. malabarica* inhibited the growth of *P. acnes* significantly (Table 3). Further, it has been demonstrated for the first time that embelin and chebulagic acid isolated from the active fractions of *T. chebula* and *E. ribes* were the most potent inhibitors of *P. acnes*. Further investigation on the actual mechanism of inhibition is being carried in our laboratory.

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