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In vitro pharmacological potential of *Epiprinus mallotiformis* – An endemic species of Western Ghats

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Epiprinus mallotiformis is an endemic species of Western Ghats, traditionally known to cure dysentery, digestive problems, ulcers, gonorrhoea and also as a good antimicrobial and diuretic agent. The present investigation was done to evaluate the *in vitro* pharmacological activities and preliminary screening for phytochemicals in leaf, stem and bark extracts. The study revealed an optimum antioxidant, anti-inflammatory and antimicrobial activities and the presence of phenolics, flavonoids and saponins in the plant extracts. The ethyl acetate fraction of leaf samples exhibited a very significant antibacterial activity against *S. aureus* with a minimum bactericidal concentration of 8 mg/mL which might be due to the presence of high saponin content (9 %) in the leaves. The investigation also suggests the possible use of *E. mallotiformis* as an antioxidant, anti-inflammatory and antibacterial agent.

Keywords: Anti-inflammatory, Antimicrobial, Antioxidant, *Epiprinus mallotiformis*, Phytochemical.

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Introduction

Plants are known for their medicinal properties from ancient times and were being used as a life-saving drugs since then due to the presence of important bioactive compounds[1]. Therefore, plants are considered as the tremendous source for the discovery of new products of medicinal value for drug development. Today, several distinct chemicals derived from plants are used as important drugs against different ailments in one or more countries around the world[2]. Several reports are available on the role of phytochemicals as antioxidants, antimicrobial, anticarcinogenic, anti-inflammatory, antidiabetic, antipyretic and hepatoprotective agents[3-5]. The Western Ghats is one of the world's "Hottest hotspots of biodiversity by means of its rich and varied flora with enormous species diversity abound in endemic taxa[6]. Very few plants from the Western Ghats have been subjected to isolation and characterization of secondary metabolites having their role in medicine and nutrition. *Epiprinus mallotiformis* (Müll.Arg.) Croizat commonly known as *Karinjikkada* in Tamil belongs to Euphorbiaceae family is a small tree, distributed in the forests of Western Ghats. The plant is traditionally used to treat dysentery, digestive problems, ulcers, gonorrhoea and as a good antimicrobial and diuretic agent[7]. The reported pharmacological properties of the *E. mallotiformis* include antinociceptive, anti-inflammatory, antioxidant, anthelminthic and antimicrobial activities[8-10]. The plant was reported to contain flavonoids, glycosides, saponins, steroids and tannins[10]. In the present study, *in vitro* antioxidant, antidiabetic, anti-inflammatory, acetylcholine esterase inhibitory and antimicrobial potential of different fractions of *E. mallotiformis* is evaluated along with the preliminary phytochemical evaluation of fractions.

Plant material

Stem, bark and leaves of *E. mallotiformis* were collected from the arboretum of Mangalore University Campus, authenticated by Dr. H. Soorya Prakash Shenoy, Principal Scientist & Head, Botany Section, Pilikula Nisargadhama, Mangalore, India (Voucher specimen No. MU/AB/BN-03). All the chemicals were of analytical grade and purchased from Merck (India), Himedia (Mumbai) and SRL (India). DPPH, acarbose, α-amylase, acetylcholine esterase and galanthamine were purchased from Sigma Aldrich (India).

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Extraction and fractionation

Shade-dried and powdered plant materials (50 g) were extracted in hot methanol using Soxhlet. Water extract of the plant materials (50 g) was collected by extracting at 60 °C water bath. Both the extracts were concentrated to dryness using a vacuum concentrator (Eppendorf, India) and stored at 0-4 °C until use. The methanol extract was further used for solvent-solvent fractionation[11]. Crude methanol extracts of stem, bark and leaves were fractionated separately into n-Hexane, chloroform, ethyl acetate and aqueous to collect different fractions from non-polar to polar. All the fractions were collected, evaporated to dryness and stored at 0-4 °C until use.

Preliminary phytochemical analysis

The preliminary phytochemical analysis was carried out to detect the presence of phenolics, flavonoids, alkaloids, terpenes and saponins in different fractions[12-14]. The quantitative determination of phenolics and flavonoids were done as per the standard protocols[13-16]. Quantitative determination of saponins was carried out according to Obadoni and Ochuko[17].

Antioxidant activity

Free radical scavenging activity

Free radical scavenging activities of the extracts were determined using 1, 1-Diphenyl-2-picryl hydrazyl radical (DPPH)[13,18]. For this, 2.5 mL of 0.3 mM ethanolic DPPH solution was added to different concentrations of plant extracts and gallic acid as a reference standard. The reaction mixture was allowed to react for 30 minutes at room temperature. Ethanol was used as blank and ethanol with DPPH without sample served as positive control. The absorbance was read at 518 nm and converted into percentage radical scavenging activity as follows.

$$\text{Scavenging activity (\%)} = [A - B] / A \times 100$$

Where, A is the absorbance of DPPH radical + ethanol, B is the absorbance of DPPH radical + extract or standard. The SC_{50} values of test samples were later calculated by the molar absorption coefficient of plots.

Reducing power assay

Reducing power of the samples was determined according to the method of Oyaizu[13,19]. Different volumes of test samples were pipetted out in to test tubes and the volume was made up to 1 mL with water. To this, 2.5 mL of 0.2 M phosphate buffer and 2.5 mL of potassium ferricyanide were added, mixed and incubated at 50 °C for 20 minutes. To, 2.5 mL of the supernatant, 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride were added, mixed and the absorbance was read at 700 nm against blank and observed the absorption pattern of the sample.

Anti-diabetic activity

In vitro antidiabetic activity was carried out by α-amyalse[20] and α- glucosidase[21] inhibitory assays. To 0.5 mg/mL of α-amylase in 50 mM phosphate buffer (pH 5.8) and 20 µg/mL of standard acarbose or test sample, 1 % starch was added as substrate. The reaction mixture was incubated at room temperature for 10 minutes, dinitrosalysilic acid was added, incubated in a boiling water bath for 5 minutes, cooled and the absorbance was recorded at 540 nm. Percent inhibition was calculated as follows,

$$\text{\% Inhibition} = [A\text{-}B/A] \times 100$$

Where A is the absorbance of the enzyme + substrate, B is the is the absorbance of the enzyme + substrate + sample

The α-glucosidase assay mixture containing 20 mU of α – glucosidase in 0.3 mM phosphate buffer (pH 7) was mixed with standard/test sample (200 uL) and incubated at 37 ºC for 10 minutes. After which 2 mM of *p*-nitro phenyl –α-D–glucopyranoside was added, incubated for 10 minutes at 37 ºC and the reaction was terminated with the addition of 0.2 M sodium carbonate. The absorbance of released *p*-nitrophenol was recorded at 405 nm using a microplate reader (Biotek Synergy H1). The reaction mixture containing a buffer in place of the standard/test sample served as positive control and the addition of sodium carbonate at the beginning served as negative control. Glucosidase inhibitory activity was calculated using the formula as given above and the IC_{50} values for α-amylase and α-glucosidase were later calculated using molar absorption coefficient of plots.

Anti-inflammatory activity

In vitro anti-inflammatory activity was carried out as per Chippada *et al.*[22] using human RBC lysis method. Fresh human blood was collected from healthy volunteers, centrifuged at 3000 rpm for 10 minutes and cell pellets were washed thrice with isosaline (0.9 % NaCl). The blood was then resuspended in isosaline (10 % v/v) and used for the studies. The assay mixture contained 1 mL of 0.15 M phosphate buffer (pH 7.4), 0.36 % hyposaline, 0.5 mL of blood and 0.5 mL of test or standard diclofenac

sodium salt at 100 and 200 µg/mL concentrations. The reaction mixture was incubated at room temperature for 10 minutes, centrifuged at 3000 rpm and the absorbance of the supernatant was measured at 540 nm. The reaction mixture containing distilled water instead of hyposaline served as control. The percent haemolysis and inhibition were calculated as follows.

% Heamolysis= Absorbance of test / Absorbance of control × 100

% inhibition= 100 - % heamolysis

Acetylcholine esterase (AChE) inhibitory activity

Modified Ellman technique[23] was followed to determine the AChE inhibitory activity. AChE (0.03 U/mL, 20 µL) was taken in sodium phosphate buffer (pH 8, 100 mM, 140 µL) and mixed with 10 µL of DTNB and 20 µL of sample. The reaction mixture was incubated for 15 minutes at 25 °C and the reaction was terminated using acetyl thiocholine iodide (0.5 mM, 10 µL). The absorbance of the yellow colour developed was recorded at 412 nm. Percent inhibition was calculated using the formula

% Inhibition= Absorbance of (control – test)/ Absorbance of control × 100

Antibacterial activity

Bacterial cultures and incubation conditions

In vitro antibacterial activity of different solvent extracts of plant samples were tested against two Gram-positive bacteria (*Staphylococcus aureus,* MTCC No. 7443; *Bacillus subtilis,* MTCC No. 2274) and three Gram-negative bacteria (*Escherichia coli,* MTCC No. 8933; *Psuedomonas aeroginosa,* MTCC No. 4637 and *Proteus vulgaris* MTCC No. 426) obtained from National Chemical Laboratory, Pune, India. 200 µL of overnight grown cultures of each organism was dispensed into 20 mL of sterile nutrient broth and incubated for 4-5 hours at 37 °C to obtain the culture of ~10^6 CFU.

Preliminary antibacterial study

Antibacterial assay was carried out by disc and well diffusion methods[24]. For disc diffusion method, 0.1 mL of bacterial culture (~10^6 CFU) was placed on Muller Hinton agar medium and spread throughout the plate by spread plate technique. The sterile discs (6 mm in diameter) purchased from Himedia Laboratories Mumbai, soaked with 10 µL of plant extract (10 mg/mL) was placed on the surface of the medium and incubated at 37 °C for 24 hours. For well diffusion assay, 6 mm wells were made on agar plates using a sterile cork borer and 100 µL of plant extract (10 mg/mL) was loaded into the wells. Antibacterial activity was recorded by measuring the diameter of the zone of inhibition. Streptomycin, penicillin and tetracyclin were used as positive reference standards and DMSO was used as a control.

Determination of MIC and MBC

Of the different fractions and bacterial cultures, only those which showed good inhibitory zones were tested for the determination of minimal inhibitory concentration (MIC). The test sample was prepared by serial dilution technique at the concentrations of 0.125-16 mg/mL and standards at the concentrations of 0.0039-0.125 mg/mL. MIC was determined by agar well diffusion assay as mentioned before by taking samples at different concentrations. The minimum concentration of the sample/standard showing zone of inhibition was considered as MIC.

The samples used for MIC were further used to determine the minimum bactericidal concentration (MBC). Test samples and standards were prepared as explained previously for the MIC. Different concentrations of samples/standards were added to tubes with 2 mL of nutrient broth containing 20 µL of cell culture. After overnight incubation at 37 °C, 100 µL of each sample from the tube which did not show any turbidity was inoculated on to nutrient agar. The plates were incubated for 24 hours at 37 °C and observed for the growth of cultures. DMSO was used as a control. The concentration at which there was no bacterial growth was considered as MBC.

Statistical analysis

All the experiments were carried out in triplicate and the data expressed as mean±standard deviation (SD). One-way ANOVA using SPSS 21 software was performed for the analysis of data and expressed at a significant level of $p < 0.05$.

Results and Discussion

Preliminary phytochemical analysis

Preliminary phytochemical analysis revealed the presence of phenolics, flavonoids, tannins, saponins and steroids in different fractions extracted from stem, bark and leaf (Table 1). However, alkaloids were not detected in any of the samples. The present result was supported by the results of *E. mallotiformis* reported

Table 1 — Phytochemical screening in different extracts of *E. mallotiformis*

Extract →	Leaf						Stem						Bark					
Phytochemical - name of the test ↓	M	W	H	C	EA	Aq	M	W	H	C	EA	Aq	M	W	H	C	EA	Aq
Alkaloid- Dragendorff's	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloid-Mayer's	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloid-Wagner's	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenolics- Maule	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-
Phenolics- Ellagic acid	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
Flavonoids-$FeCl_3$	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-
Flavonoids-lead acetate	+	+	-	-	+	+	+	+	-	-	+	-	+	-	-	+	+	+
Flavonoids-Shinoda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids – Zn/HCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannin- $FeCl_3$	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-
Tannin- gelatin	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Steroids - Salkowski	-	-	+	+	-	-	-	-	+	+	-	-	+	-	+	+	-	-
Steroid-Libermann-Burchard	-	-	+	+	-	-	-	-	+	+	-	-	+	-	+	+	-	-
Saponin-foam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponin- hemolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

- = absent, + = present
M= Methanol, W= Water, H= Hexane, C= Chloroform, EA= Ethyl acetate, Aq= Aqueous

Table 2 — Total phenol, flavonoid contents and antioxidant activity of *E. mallotiformis*

Sl No.	Plant part/standard	Phenol content # *	Flavonoid content $ *	Antioxidant SC_{50} £*
1.	Leaf methanol	111.67 ± 2.89^{b}	12.90 ± 1.93^{a}	0.066^{ab}
2.	Leaf water	17.50 ± 2.50^{e}	0.0^{c}	0.633^{i}
3.	Leaf hexane fraction	30.00 ± 5.00^{d}	0.0^{c}	0.533^{h}
4.	Leaf chloroform fraction	42.50 ± 4.33^{c}	0.0^{c}	1.566^{l}
5.	Leaf ethyl acetate fraction	219.17 ± 8.78^{a}	12.50 ± 2.50^{a}	0.046^{ab}
6.	Leaf aqueous fraction	37.50 ± 9.01^{c}	0.0^{c}	0.306^{ef}
7.	Stem methanol	3.33 ± 0.58^{h}	5.00 ± 1.00^{b}	1.686^{m}
8.	Stem water	13.00 ± 0.00^{efg}	5.67 ± 0.58^{b}	0.573^{hi}
9.	Stem hexane fraction	6.33 ± 0.58^{gh}	0.0^{c}	1.960^{n}
10.	Stem chloroform fraction	13.33 ± 0.58^{efg}	4.67 ± 0.58^{b}	0.633^{i}
11.	Stem ethyl acetate fraction	27.00 ± 1.00^{d}	5.67 ± 0.58^{b}	0.173^{c}
12.	Stem aqueous fraction	6.33 ± 0.58^{gh}	0.0^{c}	0.953^{j}
13.	Bark methanol	28.33 ± 3.22^{d}	6.00 ± 1.00^{b}	0.113^{bc}
14.	Bark water	17.67 ± 3.22^{e}	5.33 ± 1.15^{b}	0.260^{d}
15.	Bark hexane fraction	8.67 ± 2.31^{fgh}	0.0^{c}	1.050^{k}
16.	Bark chloroform fraction	15.33 ± 2.52^{ef}	5.67 ± 1.15^{b}	0.370^{f}
17.	Bark ethyl acetate fraction	42.67 ± 2.08^{c}	5.33 ± 0.58^{b}	0.042^{ab}
18.	Bark aqueous fraction	9.67 ± 0.58^{fgh}	0.0^{c}	0.460^{g}
19.	Gallic acid	-	-	0.016^{a}

mg gallic acid equivalent/g of sample, $ mg quercetin equivalent/g of sample, £ mg/mL
* Values are the means of three experiments± standard deviation. Values with different alphabets indicate significant difference at 5 % level.

by Chandrashekar *et al*.[8] and Chandrashekar and Naika[10]. Ethyl acetate fraction from the crude methanol extract of leaf showed a highest phenolic (219.17±8.78 mg/g) and flavonoid (12.50±2.50 mg/g) contents (Table 2). The methanol extract of the leaf also showed an on par flavonoid content (12.90±1.93 mg/g) with ethyl acetate fraction of leaf. Further, the samples showed significantly high saponin content in leaf (yield 9 %), stem (yield 6.4 %) and bark (yield 6.3 %). Phenolics and flavonoids are the most abundantly distributed phytochemicals in plants with one or more aromatic rings and hydroxyl groups[25] and act as good antioxidants by preventing oxidative stress associated with various diseases such as cancer, cardiovascular and neurodegenerative diseases[26]. Saponins with haemolytic properties are known to

lower blood cholesterol level and also act as anticancer and antimicrobial agents[27,28]. In addition, natural products exhibit reduced side effects compared to synthetic drugs[29]. Hence, the preliminary findings on the phenolics, flavonoids and steroids in *E. mallotiformis* may be explored further for the extraction of one or more bioactive molecule.

Antioxidant activity

Reducing power and DPPH radical scavenging assays are the *in vitro* methods to determine the antioxidant activity of plant extracts[30]. Reducing power assay of *E. mallotiformis* showed an increase in absorption with increased concentration of the extract indicating the antioxidant activity of the plant. Ethyl acetate extract of bark and leaf samples and methanol extract of leaf samples exhibited a good antioxidant activity with SC_{50} value ranging between 0.042 mg/mL to 0.066 mg/mL (Table 2). Increased antioxidant activity in ethyl acetate and methanol extract of *E. mallotiformis* may be due to the higher content of phenolics and flavonoids in these samples. Our result is comparable with DPPH radical scavenging activity of methanol extract of *E. mallotiformis*[9]. However, linear regression analysis failed to show a direct correlation between the total phenolic contents in the extracts and their SC_{50} values (Fig. 1a). The hydroxyl groups in the phenolics and flavonoids are responsible for the reduction of DPPH molecules and hence, extracts with more hydroxyl groups will show higher antioxidant activity[16,31]. In the present study, the absence of correlation between the phenolic contents with the scavenging of DPPH radical might be due to the presence of phenolics with less number of hydroxyl groups as reported in sweet *Citrus sinensis*[32] and in berry and fruit wines and liquors[33]. Plant phenolics and flavonoids act as good antioxidants with reduced side effects and, protect cells/tissues from inflammation and chemotherapy-induced damages[34-39]. Therefore, the antioxidant property of *E. mallotiformis* may also be studied further in the treatment of diseases such as cancer.

Anti-diabetic activity

Inhibition of α– amylase and α– glucosidase is necessary to prevent the hydrolysis of starch and disaccharides thus reducing the availability of free glucose to tissues. Phytochemicals with α – amylase and α–glucosidase inhibitory activity are used as potent anti-diabetic drugs with reduced side effects[40]. In the present investigation, *E. mallotiformis* extracts and fractions did not show good anti-diabetic activity

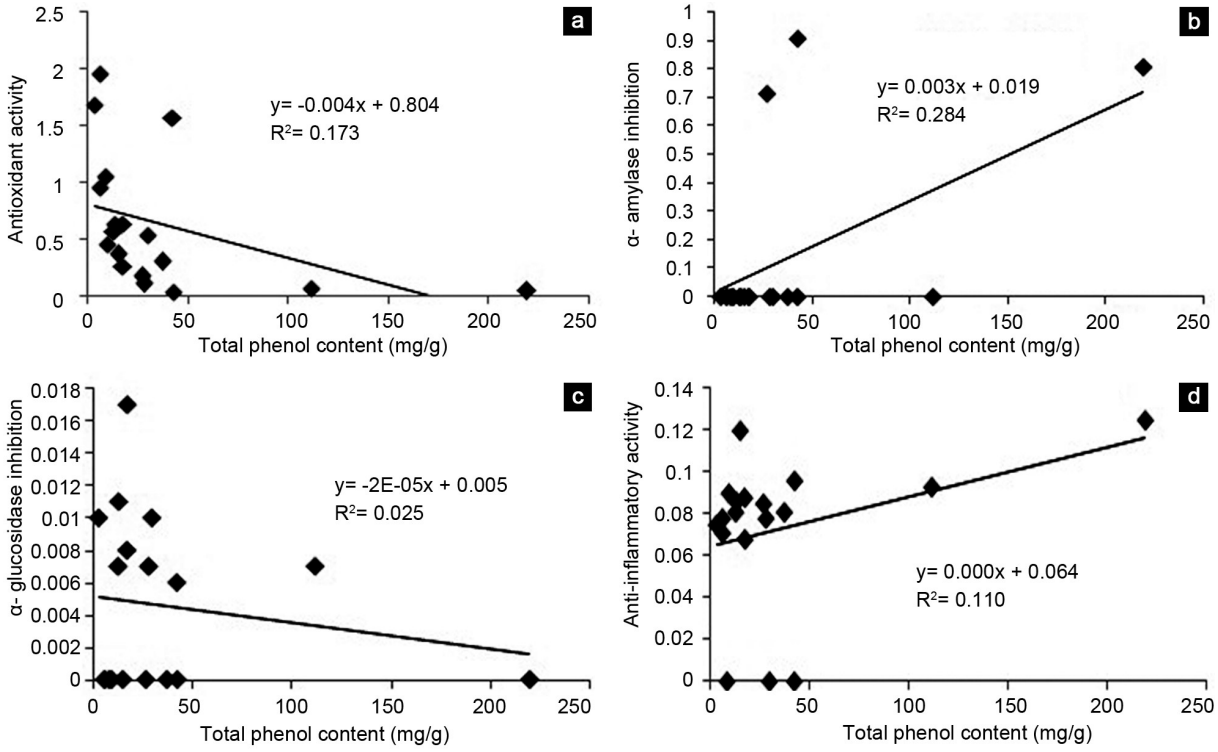

Fig. 1 — Correlation between bioactivity and total phenol content in different extracts of *E. mallotiformis*. a) Correlation between antioxidant activity and total phenol, b) and c) Correlation between antidiabetic activity and total phenol, d) - Correlation between anti-inflammatory activity and total phenol.

under *in vitro* (Table 3). However, among the samples tested, only ethyl acetate fractions showed α– amylase inhibitory activity and leaf chloroform, leaf methanol, stem water and bark methanol extracts showed comparatively higher α–glucosidase inhibitory activity with negative correlation to total phenols (Fig. 1 b and c).

Anti-inflammatory activity

Inflammation is a protective measure towards tissue damage involving an array of signalling mechanisms including the release of mediators, enzyme activation, cell migration, tissue damage and repair[41, 42]. In the present study, except leaf methanol, bark chloroform, ethyl acetate fractions of leaf and bark all other samples exhibited a significant HRBC membrane stabilisation activity with SC_{50} values between 0.068-0.088 mg/mL and was on par with standard sodium diclofenac (SC_{50}= 0.065 mg/mL) (Table 3) with negative correlation to total phenol (Fig. 1d). The result obtained is in support of the previous report on the anti-inflammatory activity of methanol extract of *E. mallotiformis* leaves on carrageenan-induced rat paw oedema models[8]. In addition, flavonoids, phenolics, alkaloids and saponins act as anti-inflammatory agents by stabilising the membrane activity to prevent the release of lysosomal enzymes and other mediators of inflammatory response[34,43,44]. Hence, further work is necessary to identify the active principle present in *E. mallotiformis* responsible for the anti-inflammatory activity.

AChE inhibitory activity

In the present study, *E. mallotiformis* extracts failed to show acetylcholine esterase inhibitory activity suggesting the absence of compounds acting on this enzyme (Table 3).

Antibacterial activity

Crude methanol extract and ethyl acetate fractions of all the samples of *E. mallotiformis* exhibited antibacterial activity against all the bacterial strains tested in the present study (Table 4). Chloroform fraction of bark sample also exhibited antibacterial activity against *E. coli*, *K. pneumonia and B. subtilis*. In both disc diffusion and well diffusion methods, ethyl acetate fraction showed comparatively better inhibition zones than methanol extract. Among the different extracts taken, leaf extract showed better inhibition than stem and bark extracts. Therefore, only methanol extract and ethyl acetate fraction of leaf sample were considered for minimum inhibitory concentration assay. The study revealed a good inhibition in the growth of *S. aureus* in culture broth compared to all other strains. The variation in the inhibitory action of different fractions might be due to the distribution of active principle in different

Table 3 — Anti-diabetic, anti-inflammtory and AchE inhibitory activities of *E. mallotiformis*

Sl No.	Plant part/standard	Inhibitory concentration - (mg/mL) *			
		α –amylase IC_{50}	α-glucosidase IC_{50}	HRBC lysis	AchE
1.	Leaf methanol	-	0.007^{bc}	0.093^{ab}	0.057^{c}
2.	Leaf water	-	0.008^{bcd}	0.088^{a}	0.038^{b}
3.	Leaf hexane fraction	-	0.010^{cd}	-	-
4.	Leaf chloroform fraction	-	0.006^{ab}	-	-
5.	Leaf ethyl acetate fraction	0.806^{b}	-	0.125^{c}	0.079^{d}
6.	Leaf aqueous fraction	-	-	0.081^{a}	0.064^{c}
7.	Stem methanol	-	0.010^{cd}	0.075^{a}	0.061^{c}
8.	Stem water	-	0.007^{bc}	0.081^{a}	-
9.	Stem hexane fraction	-	-	0.078^{a}	-
10.	Stem chloroform fraction	-	0.011^{d}	0.086^{a}	-
11.	Stem ethyl acetate fraction	0.713^{b}	-	0.085^{a}	-
12.	Stem aqueous fraction	-	-	0.071^{a}	0.062^{c}
13.	Bark methanol	-	0.007^{bc}	0.078^{a}	0.062^{c}
14.	Bark water	-	0.017^{e}	0.068^{a}	0.068^{cd}
15.	Bark hexane fraction	-	-	-	-
16.	Bark chloroform fraction	-	-	0.120^{bc}	-
17.	Bark ethyl acetate fraction	0.906^{b}	-	0.096^{ab}	-
18.	Bark aqueous fraction	-	-	0.090^{a}	-
19.	Acarbose	0.328^{a}	0.003^{a}		
20.	Galanthamine				0.00007^{a}
21.	Sodium diclofenac			0.065^{a}	

* Values are the means of three experiments. Values with different alphabets indicate significant difference at 5 % level.

fractions. Also, the results exhibited a significant antibacterial activity in mid-polar solvents like ethyl acetate compared to organic solvents. Furthermore, the compounds acting against bacterial strains are absent in water extract and aqueous fractions. This clearly indicates the mid-polar nature of compounds acting against the bacteria. MIC of leaf methanol extract was higher (0.5 mg/mL) than leaf ethyl acetate fraction (0.25 mg/mL) (Table 5) against *S. aureus*. Similarly, MBC of leaf methanol and leaf ethyl acetate fractions were >16 mg/mL and 8 mg/mL respectively. Penicillin and tetracyclin showed MIC at 0.0078 mg/mL whereas streptomycin showed MIC at 0.0312 mg/mL concentration. However, MBC of penicillin was higher (0.25 mg/mL) than streptomycin and tetracyclin (0.125 mg/mL). Hence, the present study confirms the presence of an antibacterial compound with a higher affinity towards ethyl acetate. Further purification of ethyl acetate fraction is needed to identify the active principle which might

Table 5 — Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *E. mallotiformis* against *S. aureus*

Standard / extract	MIC* – mg/mL	MBC* – mg/mL
Streptomycin	0.0312	0.125
Penicillin	0.0078	0.25
Tetracycline	0.0078	0.125
Leaf methanol extract	0.5	>16.0
Leaf ethyl acetate extract	0.25	8.0

* Values are the means of three experiments

Table 4 — Antibacterial activity of *E. mallotiformis* against selected bacterial strains

		Inhibitions zones (mm)*								
Extracts		Meth	Wat	Hex	Chlor	EA	Aqu	Strept	Penic	Tetrac
E. coli	Leaf	0.9±0.1	-	-	-	1.3±0.2	-	0.8±0.2	1.8±0.2	2.8±0.2
	Stem	0.8±0.1	-	-	-	1.0±0.1	-			
	Bark	0.9±0.2	-	-	0.9±0.1	1.2±0.2	-			
B. subtilis	Leaf	1.0±0.1	-	-	-	1.2±0.3	-	2.1±0.4	3.8±0.4	2.7±0
	Stem	0.9±0.1	-	-	-	0.9±0.2	-			
	Bark	0.8±0.1	-	-	1.2±0.1	1.1±0.1	-			
K. pneumoniae	Leaf	0.9±0.2	-	-	-	1.1±0.1	-	1.3±0.2	1.0±0.0	2.7±1.1
	Stem	0.8±0.2	-	-	-	1.0±0.2	-			
	Bark	0.8±0.2	-	-	0.7±0.2	1.1±0.1	-			
P. vulgaris	Leaf	0.9±0.2	-	-	-	1.2±0.1	-	-	1.0±0.2	2.3±0.3
	Stem	0.8±0.1	-	-	-	1.0±0.3	-			
	Bark	1.0±0.1	-	-	-	1.0±0.2	-			
S. aureus	Leaf	0.9±0.1	-	-	-	1.2±0.3	-	1.1±0.2	1.4±0.1	2.6±0.7
	Stem	0.8±0.2	-	-	-	1.1±0.2	-			
	Bark	1.0±0.2	-	-	-	1.1±0.1	-			
P aeroginosa	Leaf	1.1±0.2	-	-	-	1.1±0.1	-	-	1.2±0.1	2.6±0.6
	Stem	1.0±0.1	-	-	-	1.1±0.1	-			
	Bark	0.8±0.1	-	-	-	0.8±0.1	-			

* Values are the means of three experiments ± standard deviation. Extract conc. 10 mg/mL, standard conc. 10 µg/mL. - = No inhibition
Meth= methanol, Wat= water, Hex= hexane, Chlor= chloroform, EA= ethyl acetate, Aqu= aqueous, Strept= streptomycin, Penic= penicillin, Tetrac= tetracyclin

enhance the activity. In the present study, both methanol extract and ethyl acetate fractions showed the presence of the highest quantity of phenols, flavonoids and saponins which might be responsible for antibacterial activity as reported in other plant species[45,46]. It may be further considered that the ethyl acetate fraction might also be used to test against hospital isolates of *S. aureus* which are resistant to methicillin drugs. Chandrashekar and Naika[10] also reported the antibacterial and antifungal activity of *E. mallotiformis*. A good antibacterial activity of *E. mallotiformis* observed in the present study is also supported by the traditional use of this plant against microbial infection[7].

Conclusion

The present study revealed the presence of high phenolic and flavonoid contents in methanol and ethyl acetate extracts of plant samples. A good yield of saponin was observed in leaf samples followed by stem and bark samples. Pharmacological evaluation of different extracts showed a significant antioxidant, anti-inflammatory and antimicrobial activity of *E. mallotiformis* in methanol and ethyl acetate extracts of plant samples which might be due to the presence of higher phenolics, flavonoids and saponins in these extracts. The present study also showed a significant antibacterial activity against *S. aureus* with a minimum bactericidal concentration of 8 mg/mL which might be due to high saponin content (9 %) in the leaves. Further study on the purification of ethyl acetate fraction may help to identify the active principle responsible for the antibacterial activity of *E. mallotiformis* and to understand the possible mechanism involved.

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