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Antimicrobial activity of fresh latex, juice and extract of *Euphorbia hirta* and *Euphorbia thymifolia* – an *in vitro* comparative study

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ABSTRACT

Medicinal plants are the potent source of biologically active compounds and have always been of great interest for the effective chemotherapeutic agents and offering a broad spectrum of activity with greater emphasis on preventive action. The present study was aimed at evaluating and comparing the antimicrobial spectrum of the fresh extract, diluted latex, fresh juice and various extracts (methanolic, ethanolic, DCM and aqueous) of Euphorbia hirta and Euphorbia thymifolia against different pathogenic strains of bacteria and fungi, in order to know the potent plant (sample), by adopting the disc diffusion method. 14 various samples and extracts of both plants were studied, among them fresh latex of Euphorbia hirta showed the excellent antimicrobial activity against Bacillus pumilus (24.98), Staphylococcus aureus (25.38), Streptococcus pneumonia (23.72), Escherichia coli (27.93), Citrobacter freundii (23.54) and Klebsiella pneumoniae (21.93), as compared with standard drug vancomycin (22.29 mm), flucloxacillin (24.65 mm), ceftriaxone (22.50 mm), ceftriaxone (22.50 mm), ciprofloxacin (22.36 mm) and levofloxacin (21.70 mm) with relative percentages of inhibition 125.63, 106.10, 111.15, 129.42, 110.82, and 102.16 respectively. The descending sequences of antimicrobial activity of various samples and extracts of Euphorbia hirta against studied microorganisms were as follow: fresh latex, fresh juice, methanolic extract, ethanolic extract, DCM extract, aqueous extract and diluted latex extract (no activity), whereas same descending sequence was also followed by the Euphorbia thymifolia against same studied microorganisms but Euphorbia thymifolia showed the less potent antimicrobial response as compared to Euphorbia hirta. The results of the present study indicate that the antimicrobial activity varies with the species of the plants and the plant material used and it also indicate that the fresh latex of the Euphorbia hirta is a potentially good candidate for the therapy of antibacterial-resistant bacteria and would therefore require further study.

Keywords: *Euphorbia hirta, Euphorbia thymifolia,* methanol extract, ethanol extract, dichloromethane extract, antimicrobial activity, disc diffusion method.

INTRODUCTION

Over the past few decades, medicinal plants have most widely been studied for the purpose of the mitigation and the treatment of various infectious diseases because microbial resistance against conventionally used synthetic antimicrobial agents is increasing with an alarming rate [1, 2, 3]. Survey study has revealed that almost all the microbes have developed resistance against all introduced antibiotics [4]. Methicillin resistant *Staphylococci*, vancomycin resistant *Enterococci*, penicillin resistant *Pneumococci* and gram –negative microbes having multi- drug resistant, are the prominent examples of the drug resistance [5]. In addition to resistance, antibiotics are associated with serious adverse effects on host including hypersensitivity, allergic response, immune-suppression and depletion of the gut normal flora [6]. There is an urgent need to mitigate and overcome the

antimicrobial resistance and hospital cross-infection [7]. Although a large number of antimicrobial agents already exist, the search for new drugs should be a continuous one since the target microorganisms often evolve new genetic variants which subsequently become resistant to existing antimicrobial agents. In Pakistan, more than hundreds medicinal plants have been reported to posses the anti-microbial activity, *Euphorbia hirta.* Linn and *Euphorbia thymifolia.* Linn are amongst of them, with traditional claims for curing pathogenic infections. *Euphorbia hirta,* (Family; Euphorbiaceae) is an herb that is distributed in many parts of the world [8, 9]. It is commonly known by many vernacular names such as; euphorbia, dudhi, dudhi khurd, cats' hair, asthma weed and pill bearing spurge [10]. The plant has been used for female disorders but is now more important in treating respiratory ailments, especially cough, coryza, bronchitis and asthma. It is also used to treat worm infestations in children and for gonorrhea, jaundice, pimples, digestive problems and tumors [11]. The plant is also widely used against diarrhea and dysentery, especially amoebic dysentery. Latex of the plant is used as ear drops and in the treatment of boils, sore and promoting wound healing [12]. *Euphorbia hirta* is well documented for its biological activities such as anthelmintic, lactagogue, antipyretic, anti-inflammation [13], antioxidant [14], antibacterial [15, 16], antifungal [17], and anticancer [14].

Euphorbia thymifolia (Family; Euphorbiaceae), known by the vernacular names of dudhi and dugdhikaa (choti dudhi). It is one of the important multipurpose species of desert and arid regions. It provides vegetative cover in hot, dry, sandy desert areas where little else grows and is an extremely hardy species [18]. The plant is bitter, acrid, thermogenic, and laxative, diuretic. It is useful in vitiated condition of constipation, ringworm, skin diseases and asthma [19, 20]. The leaves and seeds are given in worm cases and in certain bowel affections of children & they are considered stimulant and laxative [20]. The Stem and leaves produce the milky juice when cut. Latex of plant is traditionally used to treat eye disorder and wounds in certain regions. Antiviral activity is proven in experiment and antimicrobial activity is reported [21]. This paper gives the comparative (quantitative and qualitative) reports on the antimicrobial activities of the fresh latex, diluted latex, fresh juice, dichloromethane (DCM), ethanol, methanol and aqueous extract of *Euphorbia hirta* and *Euphorbia thymifolia*.

MATERIALS AND METHODS

Collection of plants

Euphorbia hirta and *Euphorbia thymifolia* were collected from the Botanical Garden and nearby fields around the of Bahauddin Zakariya University, Multan and were authenticated by Professor Dr. Altaf Dasti, taxonomist and head of herbarium of Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan (Pakistan). Fresh plants were collected in the due free morning and were dried under the shade for the 3 weeks. At the time of collection, weight of fresh *Euphorbia hirta* and *Euphorbia thymifolia* (separate) was almost one kg, after drying under shade weight of both plants got reduced to 750 gm and 700 gram respectively.

Extract preparation

Adulterants free dried plants (leaves, seeds and stems) were powdered in the electrical blenders. Extraction of coarse powdered material (# 40) was performed by the triple maceration [22, 23], by using the different organic solvents depending upon their polarity. Extract was prepared by soaking 400g of the coarse powdered material of *Euphorbia hirta* and *Euphorbia thymifolia* in a measured volume of dichloromethane, in two separate macerating bottles and agitated at 120 rpm/min for 72 hrs in rotary orbital shaker, at room temperature and this procedure was adopted three times with dichloromethane. After maceration, the soaked coarse powdered material was passed through muslin cloth (double layered), in order to remove vegetative debris and the obtained filtrate was subsequently filtered through a Whatman-1 filter paper. The filtrates were stored in amber glass air-tight bottles. The extraction of marc was carried out with ethanol, methanol and then with distilled water by adopting same procedure. Rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) attached with a vacuum pump and a recirculation chiller was used to concentrate the dichloromethane, ethanol and methanol extracts, under reduced pressure at 37 °C. While aqueous extract was concentrated by the lyophilization process. All extracts were stored at −4 °C in a refrigerator.

Fresh latex of the both plants was collected from the stems early in the morning by the capillary action, by using two separate amber glass screw cap bottle (almost 4 ml). For the purpose of collection of fresh juice, fresh plants were collected, rinsed with distilled water and minced slightly and juice was obtained by the squeezing the mass with the fingers. Collected juice was filtered through the muslin cloths and subjected immediately for the antimicrobial activity.

Extract solution preparation

In vitro, experiments were performed by dissolving 0.5 gram of the crude extract in 0.1ml (100 µl) of 100% dimethylsulfoxide (DMSO) and volume was made up to 1 ml (1000 µl) with distill water to prepare 0.5 g /ml, w/v stock solution (500 mg/ml), due to its insolubility in distilled water and stored in refrigerator [24]. The dimethylsulfoxide alone did not show any biological and physiological activity. Thereafter serial dilution of stock solution (containing 500 mg/ml) was made, to obtain 50 mg/ml concentration which was used for the antimicrobial sensitivity test. Diluted latex was prepared by dissolving 1.0 ml of the collected latex with ten time of distilled water, which was also used the antimicrobial sensitivity.

Determination of phytochemical constituents

Crude methanolic extracts of *Euphorbia hirta* (EhM.Cr) and *Euphorbia thymifolia* (EtM.Cr) were subjected to phytochemical screening for the detection of alkaloids, carbohydrates, tannins, saponins, anthraquinones, steroids and flavonoids as possible important constituents of the plant, according to standard method [23]. Appearance of yellowish brown coloration on mixing of Dragendorff's reagent with HCl treated aqueous plant extract solution, conform the presence of alkaloids in extract. Molisch's, benedict's and fehling's tests were performed for the detection of carbohydrates. Formation of froth on vigorous shaking of the aqueous extract solution, conform the presence of saponin. Development of blue green or dark green coloration on mixing of aqueous $FeCl_3$ with extract solution indicated presence of phenols and tannins. The appearance of pink, violet or red coloration on exposure to NH_4OH of the mixture of benzene with aqueous solution of plant extract already acidified with 1% HCl was taken as presence of anthraquinones among the plant constituents. The plant material was deemed positive for flavonoids when it gave a yellow color with $AlCl_3$ reagent.

Standard discs and micro-organisms used

All standard drug discs i.e., flucloxacillin, vancomycin, ceftriaxone, ciprofloxacin, ceftriaxone, levofloxacin and amphotericin-B, having drug concentration of 10 µg/disc (Oxobid Ltd. Basingstoke, Hampshire, England) were purchased from G.M, Scientific shop, Multan, Pakistan. While, all the microorganisms i.e., *Staphylococcus aureus, Bacillus pumilus, Streptococcus pneumoniae,* C*itrobacter freundii, Escherichia coli, Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus niger,* used for the detection of antimicrobial activity were collected from the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. All microbes were cultured overnight in a nutrient agar (pH 5) containing agar (1.2%), peptone (0.5%), yeast (0.3%), and NaCl (0.8%), [24, 25]. Inoculums were prepared by transferring microbial colonies from fresh culture plates to tube containing 10 ml of nutrient broth media. The tubes were shaken occasionally for aeration to promote the microbial growth and were incubated overnight at 37 ^{0}C.

Determination of antimicrobial activity

For the determination of the antimicrobial activity, standard disc diffusion method was adopted [24, 26, 27] and three types of discs were used, i.e., discs containing standard antibiotics were used as positive control, discs containing plant crude extract or latex were used as sample discs, and discs containing the DMSO were used as negative control. Punch machine was used to prepare the discs having the diameter of 6 mm from the whatman-1 filter. All the glass wares were sterilized by the dry heat of sterilization. Nutrient agar media and sabouraud dextrose agar media prepared in distilled water and sterilized in autoclave at 121 ^{o}C for 30 minutes. Pour the media into separate petri dishes and allowed to set as a firm gel on cooling. The thickness of gels layer should range between 2-3 mm. The test petri-dishes were incubated overnight at 37 ^{o}C and those showing no growth were selected for further work. The bacterial culture and fungal culture were transferred from inoculums to petri-dishes by using the sterilized aluminum wire loops, which were subsequently spread by streaking method. All the procedure was carried out in the strict aseptic condition using horizontal laminar flow cabinet. Bacterial cultures were incubated at 37 ^{o}C in incubator for 24 hours while fungal cultures at room temperature for 48 hours. At the end of the incubation period, zone of inhibition (mm) of the each extract was measured in comparison with the positive and negative control [28, 29]. For the conformation of the results, each test was performed in triplicate as per table.

Relative percentage inhibition

The relative percentage inhibition of the crude extract with respect to positive control was calculated by using the following formula [24, 30]

$$\textbf{Relative percentage inhibition} = \frac{100 \times (a - b)}{(c - b)}$$

Where,

a: total area of inhibition of the test extract

b: total area of inhibition of the solvent

c: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using

Area of inhibitory zone = πr^2

Where r is radius of zone of inhibition

Determination of minimum inhibitory concentration (MIC)

Modified agar well diffusion method was adopted to measure the MIC of fresh latex and methanolic crude extract of *Euphorbia hirta* (EhM.Cr) and *Euphorbia thymifolia* (EtM.Cr), [31, 32]. The crude extracts were dissolved in DMSO to obtain a concentration range of 30, 60, 90, 150, 300, 600, 1200, 2000 and 5000 µg/ml. Wells were cut out by using a cork borer. Using a micropipette, 100 µl of each dilution was added in to wells and plates were incubated at 37ºC for 24 hours. The minimum concentration of each extract showing a clear zone of inhibition was considered to be MIC.

Statistical analysis

The results of the antimicrobial activity of crude extract are expressed as mean ± standard deviation of the response of 3 replicates determinations per sample. Statistically significant differences between groups were measured using one-way analysis of variance (ANOVA) followed by two sample *t*-test of all groups versus their respective control group and $*p < 0.05$ was considered statistically significant, $p > 0.05$ was considered as non-significant and $**p < 0.01$ was considered highly significant. Results were analyzed statically by using "Graph pad Prism" version 6, (Graph Pad Software, San Diego, CA, USA).

Table 1. Phytochemical evaluation of methanolic extract of *Euphorbia hirta and Euphorbia thymifolia*

Chemical tests	EhM.Cr	EtM.Cr	Chemical tests	EhM.Cr	EtM.Cr
Test for carbohydrates			**Test for tannin**		
A. Molisch's test			FeCl3 test		
B. Fehling's test	Positive	Positive	Acetic acid test	Positive	Positive
C. Benedict's test			KmnO4 test		
Test for alkaloids			**Test for cardiac glycosides**		
A. Hager's test	Positive	Positive	Legal test	Negative	Negative
B. Wagner's test			Keller-killiani test		
C. Dragendorff's test					
Test for flavonoids			**Test for anthraquinone**	Positive	Positive
Lead acetate test	Positive	Positive	Borntragers's test		
Test for saponins			**Test for steroids**	Negative	Negative
Foam test	Positive	Positive	Lieberman burchard test		

EhM.Cr = Methanolic crude extract of *Euphorbia hirta.;* EtM.Cr = Methanolic crude extract of *Euphorbia thymifolia.*

RESULTS AND DISCUSSION

Phytochemical screening

Freshly prepared methanolic extracts of *Euphorbia hirta* (EhM.Cr) and *Euphorbia thymifolia* (EtM.Cr), were subjected to a preliminary phytochemical screening for various constituents and their results are depicted in Table 1.

In Vitro antimicrobial activity

Diameter of the zone of inhibition and relative percentage of inhibition of the dichloromethane (DCM), ethanolic extract, methanolic extract, aqueous extract, fresh latex, diluted latex and fresh juice of *Euphorbia hirta* and *Euphorbia thymifolia,* against different pathogenic bacteria and fungi species*,* are shown in tables 2, 3, 4 and 5.

Fresh latex of *Euphorbia hirta* (EhFL) showed the diameter of the zone of inhibition (including diameter of disc 6 mm) of 24.98 mm against *Bacillus pumilus*, 25.38 mm against *Staphylococcus aureus,* 23.72 mm against *Streptococcus pneumoniae,* 27.93 mm against *Escherichia coli*, 23.54 mm against *Citrobacter freundii* and 21.93 mm against *Klebsiella pneumoniae as* compared with standard drug vancomycin (22.29 mm), flucloxacillin (24.65 mm), ceftriaxone (22.50 mm), ceftriaxone (22.50 mm), ciprofloxacin (22.36 mm) and levofloxacin (21.70 mm) with relative percentages of inhibition 125.63, 106.10, 111.15, 129.42, 110.82, and 102.16 respectively, whereas, fresh latex of *Euphorbia thymifolia* (EtFL) showed the diameter of the zone of inhibition (including diameter of disc 6 mm) of 20.37 mm against *Bacillus pumilus*, 22.82 mm against *Staphylococcus aureus,* 20.94 mm against *Streptococcus pneumoniae,* 23.74 mm against *Escherichia coli*, 18.82 mm against *Citrobacter freundii* and 18.32 mm against *Klebsiella pneumoniae*, with relative percentages of inhibition 83.50, 85.75, 86.62, 93.53, 70.85 and 71.30 respectively, while the diluted latex (1: 10) of the both plants showed no antimicrobial response.

Fresh juice of *Euphorbia hirta* (EhFL) showed the relative percentages of inhibition 96.74, 88.32, 92.35, 96.75, 85.54, and 84.09 whereas fresh juice of *Euphorbia thymifolia* (EtFL) showed the relative percentages of inhibition 75.96, 75.25, 69.23, 78.75, 59.77 and 65.37 against *Bacillus pumilus*, *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli*, *Citrobacter freundii* and *Klebsiella pneumoniae* respectively.

Table 2. Zone of inhibition of the DCM, ethanolic, methanolic and aqueous extract of the *Euphorbia hirta* and *Euphorbia thymifolia* against different strains of bacteria and fungi.

Microbes used	Zone of Inhibition (mm/sensitive strain)										
	EhD.Cr[a]	EhE.Cr[a]	EhM.Cr[a]	EhAq.Cr[a]	EtD.Cr[a]	EtE.Cr[a]	EtM.Cr[a]	EtAq.Cr[a]	+ve control (10 µg/disc)		-ve
Bacillus pumilus	18.90	19.25	20.35	10.34	14.63	16.95	18.17	7.54	Vancomycin	22.29	_
Staphylococcus aureus	20.15	21.15	22.85	12.65	16.84	18.56	20.24	9.55	Flucloxacillin	24.65	_
Streptococcus pneumoniae	16.55	17.80	20.25	9.44	14.36	16.45	17.80	7.23	Ceftriaxone	22.50	_
Escherichia coli	21.45	22.80	23.55	11.56	17.84	19.23	20.65	10.66	Ceftriaxone	24.55	_
Citrobacter freundii	16.94	17.90	19.60	9.35	13.56	14.87	16.90	8.55	Ciprofloxacin	22.36	_
Klebsiella pneumoniae	17.10	17.55	18.90	9.52	12.82	14.95	16.73	9.44	Levofloxacin	21.70	_
Candida albicans	15.15	16.15	17.90	9.44	11.49	12.85	14.64	8.34	Amphotericin-B	22.35	_
Aspergillus niger	14.22	15.25	16.75	9.55	11.54	12.15	14.75	7.88	Amphotericin-B	21.85	_

Values are presented as mean of triplicate experiments,

[a] Diameter of the zone of inhibition including diameter of disc 6mm.

-ve = negative control, i.e., Dimethylsulfoxide, +ve control = standard drug discs, - = no response

EhD.Cr = crude extract of the *Euphorbia hirta* in DCM, EhE.Cr= crude extract of the *Euphorbia hirta* in ethanol, EhM.Cr= crude extract of the *Euphorbia hirta* in methanol, EhAq.Cr= Aqueous crude extract of *Euphorbia hirta*, EtD.Cr = crude extract of the *Euphorbia thymifolia* in DCM, EtE.Cr= crude extract of the *Euphorbia thymifolia* in ethanol, EtM.Cr= crude extract of the *Euphorbia thymifolia* in methanol, EtAq.Cr= Aqueous crude extract of *Euphorbia thymifolia.*

Table 3. Zone of inhibition of the fresh latex, fresh juice and diluted latex of the *Euphorbia hirta* and *Euphorbia thymifolia* against different strains of bacteria and fungi.

S.No	Microbes used	-ve	Zone of Inhibition (mm/sensitive strain)							
			Euphorbia hirta			*Euphorbia thymifolia*			Positive control	
			FL[a]	FJ[a]	DL[a]	FL[a]	FJ[a]	DL[a]	Standard discs	
1	*Bacillus pumilus*	NR	24.98	21.92	NR	20.37	19.43	NR	Vancomycin	22.29
2	*Staphylococcus aureus*	NR	25.38	23.16	NR	22.82	21.38	NR	Flucloxacillin	24.65
3	*Streptococcus pneumoniae*	NR	23.72	21.62	NR	20.94	18.72	NR	Ceftriaxone	22.50
4	*Escherichia coli*	NR	27.93	24.15	NR	23.74	21.78	NR	Ceftriaxone	24.55
5	*Citrobacter freundii*	NR	23.54	20.68	NR	18.82	17.29	NR	Ciprofloxacin	22.36
6	*Klebsiella pneumoniae*	NR	21.93	19.90	NR	18.32	17.55	NR	Levofloxacin	21.70
7	*Candida albicans*	NR	19.38	18.37	NR	16.84	15.92	NR	Amphotericin-B	22.35
8	*Aspergillus niger niger*	NR	18.68	17.38	NR	16.92	15.12	NR	Amphotericin-B	21.85

Values are presented as mean of triplicate experiments,

[a] Diameter of the zone of inhibition including diameter of disc 6mm.

-ve = negative control, i.e., Dimethylsulfoxide, FL = fresh latex, FJ = fresh juice, DL = diluted latex

Table 4. Relative percentage inhibition of DCM, ethanolic, methanolic and aqueous extract of the *Euphorbia hirta* and *Euphorbia thymifolia* against different strains of bacteria and fungi.

Microbes used	Relative percentage inhibition (%)							
	EhD.Cr	EhE.Cr	EhM.Cr	EhAq.Cr	EtD.Cr	EtE.Cr	EtM.Cr	EtAq.Cr
Bacillus pumilus	71.91	74.66	83.41	21.55	43.10	57.87	66.50	11.44
Staphylococcus aureus	76.00	73.65	86.00	26.32	46.70	56.70	67.45	15.05
Streptococcus pneumoniae	54.15	62.60	81.05	17.60	40.75	53.42	62.60	10.30
Escherichia coli	76.35	86.28	92.00	22.17	52.82	61.40	70.70	17.40
Citrobacter freundii	57.40	64.10	76.85	17.50	36.77	44.32	57.15	14.75
Klebsiella pneumoniae	62.21	65.44	75.85	19.24	34.90	47.43	59.40	18.92
Candida albicans	45.95	52.23	64.20	17.85	26.47	32.97	42.92	13.93
Aspergillus niger	42.40	48.78	58.75	19.10	27.90	30.91	47.18	48.75

Table 5. Relative percentage inhibition of fresh latex, fresh juice and diluted latex of the *Euphorbia hirta* and *Euphorbia thymifolia* against different strains of bacteria and fungi.

Microbes used	Relative percentage inhibition (%)					
	Euphorbia hirta			*Euphorbia thymifolia*		
	FL	FJ	DL	FL	FJ	DL
Bacillus pumilus	125.63	96.74	NR	83.50	75.96	NR
Staphylococcus aureus	106.10	88.32	NR	85.75	75.25	NR
Streptococcus pneumoniae	111.15	92.35	NR	86.62	69.23	NR
Escherichia coli	129.42	96.75	NR	93.53	78.75	NR
Citrobacter freundii	110.82	85.54	NR	70.85	59.77	NR
Klebsiella pneumoniae	102.16	84.09	NR	71.30	65.37	NR
Candida albicans	75.22	67.55	NR	56.80	50.76	NR
Aspergillus niger	73.14	63.40	NR	60.0	47.90	NR

FL = fresh latex, FJ = fresh juice, DL = diluted latex.

Methanolic extract of *Euphorbia hirta* and *Euphorbia thymifolia* showed the zone of inhibition of 23.55 mm and 20.65 mm against *Escherichia coli*, as compared with standard drug Ceftriaxone (24.55 mm), with relative percentages of inhibition 92.00 and 70.70 respectively, whereas ethanolic extract of *Euphorbia hirta* and *Euphorbia thymifolia* showed the zone of inhibition of 22.80 mm and 19.23 mm against *Escherichia coli*, as compared with standard drug Ceftriaxone (24.55 mm), with relative percentages of inhibition 86.28 and 61.40 respectively. DCM and aqueous extracts of both plants showed the weaker antibacterial response against studied microbes as compared to ethanolic and methanolic extracts. Whereas, fresh latex, juice and various extracts (methanolic, ethanolic, DCM, aqueous), of both plants showed poor inhibitory response against *Candida albicans* and *Aspergillus niger,* as compared to standard antifungal drug, i.e., Amphotericin-B.

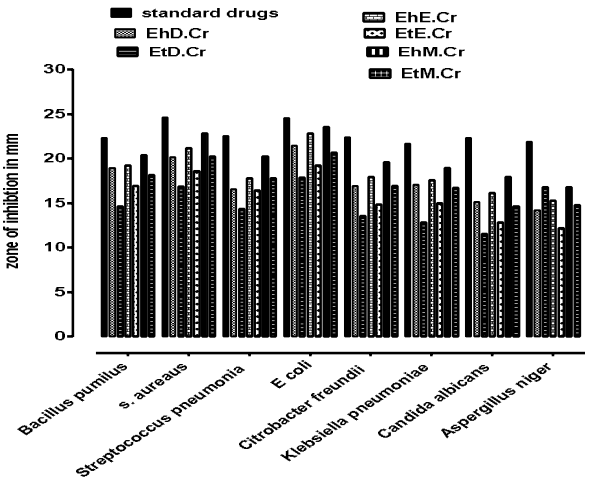
It can be inferred that fresh latex and juice of *Euphorbia hirta* showed excellent antibacterial activity against studied pathogenic microbes, as compared to fresh latex and juice of *Euphorbia thymifolia* whereas, among the extracts, methanolic and ethanolic extract of *Euphorbia hirta* showed good antibacterial response as

compares to methanolic and ethanolic extract of *Euphorbia thymifolia*. After statistical analysis, P value was determined which was found to be significant for fresh latex and fresh juice of both plants, i.e., less than 0.05 ($P < 0.05$). It shows that fresh latex and fresh juice of both plants have potent antibacterial activity against studied microbes as compared to extracts of plants (methanolic, ethanolic, DCM and aqueous), while very weak response against fungal species.

Minimum inhibitory concentration

As shown in Table 6, fresh latex of the *Euphorbia hirta* showed strong inhibition against tested G +ve and G – ve bacteria with MIC value of 30 µg/ml, whereas, MIC values for the methanolic extract of *Euphorbia hirta* were ranged from 60-150 µg/ml. For the fresh latex and methanolic extract of the *Euphorbia thymifolia*, MIC values were ranged from 60-150 and 90-300 µg/ml respectively. MIC values for the fungal species were ranged from 600-2000 µg/ml.

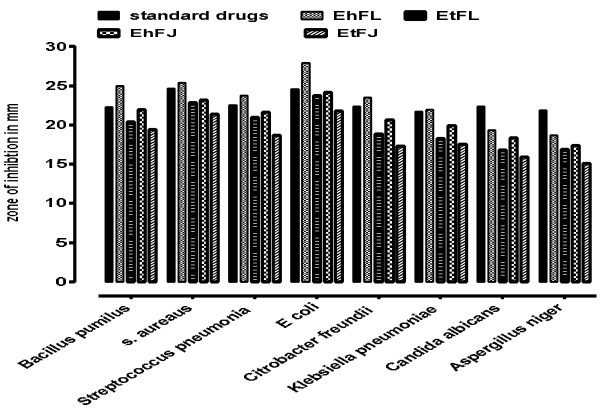
In this study, fresh latex of the *Euphorbia hirta* showed the highest antibacterial activity against the bacteria tested with lowest MIC values of 30 µg/ml.



(A)

EhD.Cr = crude extract of the *Euphorbia hirta* in DCM, EhE.Cr= crude extract of the *Euphorbia hirta* in ethanol, EhM.Cr= crude extract of the *Euphorbia hirta* in methanol, EtD.Cr = crude extract of the *Euphorbia thymifolia* in DCM, EtE.Cr= crude extract of the *Euphorbia thymifolia* in ethanol, EtM.Cr= crude extract of the *Euphorbia thymifolia* in methanol.

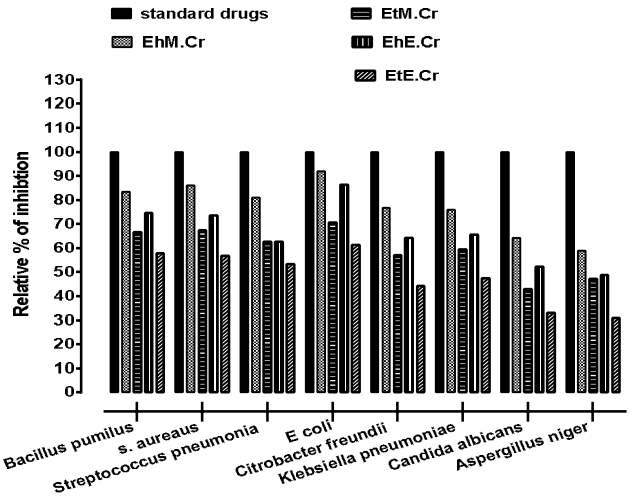
(B)



EhFL = fresh latex of *Euphorbia hirta*, EtFL = fresh latex of *Euphorbia thymifolia*

Eh FJ = fresh juice of *Euphorbia hirta*, EtFJ = fresh juice of *Euphorbia thymifolia*

Figure 1. Zone of inhibition of (A) DCM, ethanolic and methanolic extract (B) fresh latex and fresh juice of *Euphorbia hirta* and *Euphorbia thymifolia* in diameter (mm) against different strains of bacteria and fungi (values are expressed as mean ± SEM., n = 3).



(A)

EhM.Cr= crude extract of the *Euphorbia hirta* in methanol, EhE.Cr= crude extract of the *Euphorbia hirta* in ethanol, EtM.Cr= crude extract of the *Euphorbia thymifolia* in methanol, EtE.Cr= crude extract of the *Euphorbia thymifolia* in ethanol.

(B)

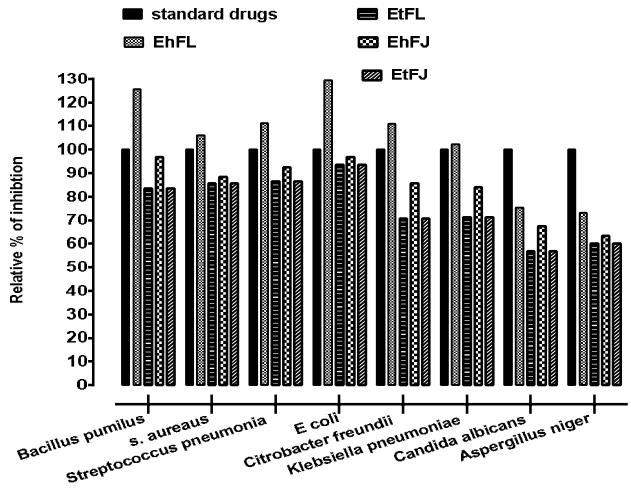


Figure 2. Relative percentage inhibition of (A) methanolic and ethanolic extract (B) fresh latex and fresh juice of *Euphorbia hirta* and *Euphorbia thymifolia* in diameter (mm) against different strains of bacteria and fungi (values are expressed as mean ± SEM., n = 3).

Table 6. Minimum inhibition concentration (MIC) against different strains of bacteria and fungi

S.No	Bacterial strains	Gram Strain (+/-)	Minimum inhibitory concentration(µg/ml)			
			Euphorbia hirta		*Euphorbia thymifolia*	
			FL	M	FL	M
1	*Bacillus pumilus*	+	30	60	60	90
2	*Staphylococcus aureus*	+	30	60	60	90
3	*Streptococcus pneumoniae*	+	30	60	60	90
4	*Escherichia coli*	_	30	60	60	150
5	*Citrobacter freundii*	_	30	90	90	300
6	*Klebsiella pneumoniae*	_	30	150	150	300
7	*Candida albicans*	fungus	600	1200	1200	2000
8	*Aspergillus niger*	fungus	600	1200	1200	2000

Where; FL = fresh latex, M = methanolic extract,

Researchers are mainly focusing to the medicinal plants, and are trying their best to develop new natural products from medicinal plants against multidrug resistant microbial strains [33]. There is an urgent need to develop new natural antimicrobial agents for human and veterinary therapeutic uses, as resistant to the current drugs are increasing day by day [34, 35]. Plants are the invaluable sources of the pharmaceutical products [36]. Secondary metabolites isolated from the medicinal plants have been reported to possess the antimicrobial property [24]. In the previous studies, antimicrobial activity of *Euphorbia hirta* and *Euphorbia thymifolia* has been studied [37, 38, 39, 40]. The present study was aimed to compare the antimicrobial spectrum of the fresh extract, diluted latex, fresh juice and different extracts (methanolic, ethanolic, DCM and aqueous) of *Euphorbia hirta* and *Euphorbia thymifolia* against different strains of bacteria and fungi, by adopting the disc diffusion method.

Our present research study results clearly indicates that the fresh latex, fresh juice and extracts (methanolic, ethanolic) of the *Euphorbia hirta* posses the potent antibacterial activity against studied pathogenic bacterial species as compared to *Euphorbia thymifolia* and support the view, that medicinal plants might be useful in the development of novel antimicrobial agents [41]. Resistance against *S. aureus* has been developed due to extensive use of antibiotic, in the management of infectious disease [24]. Fresh latex of *Euphorbia hirta* has showed significant antibacterial activity with respect to all other sample used against different pathogenic species of G +ve bacteria, i.e., *Bacillus pumilus, Staphylococcus aureus, and Streptococcus pneumoniae,* with relative percentage of inhibition of 125.63 %, 106.10 %, and 111.15 % respectively, as compared with standard vancomycin, flucloxacillin and ceftriaxone (10 µg/disc), respectively. Multidrug resistant bacteria have limited the efficacy of antibiotics against infections caused by the G –ve bacteria. In earlier studies it has been found that G-ve are less susceptible to plant extracts [42] but in our study, we observed that fresh latex of Euphorbia hirta was more potent against different pathogenic species of G –ve bacteria, i.e., *Escherichia coli,* C*itrobacter freundii,* and *Klebsiella pneumoniae,* with relative percentage of inhibition of 129.42 %, 110.82 %, and 102.16 % respectively, as compared with standard, i.e., ceftriaxone, ciprofloxacin and levofloxacin (10 µg/disc), respectively. Similarly, fresh latex of *Euphorbia thymifolia* showed the good antibacterial activity against studied microbes but less than *Euphorbia hirta*. The descending sequences of antimicrobial activity of various samples and extracts of *Euphorbia hirta* against studied microorganisms were as follow: fresh latex, fresh juice, methanolic extract, ethanolic extract, DCM extract, aqueous extract and diluted latex extract, whereas same descending sequence was also followed by the various samples and extracts of *Euphorbia thymifolia* against same studied microorganisms but *Euphorbia thymifolia* showed the less potent antimicrobial response as compared to *Euphorbia hirta*.

CONCLUSION

Euphorbia hirta is believed to possess the strong antibacterial activity than *Euphorbia thymifolia* due to presence of tannin, alkaloids and flavonoids which have been studied [43]. Tannins have important role such as stable and potent antioxidants [44]. Most of the organisms used in the research study were causative agents of diarrhea and dysentery, while *Euphorbia hirta* and *Euphorbia thymifolia* inhibit the growth of these microbes, so both plants can be used for the treatment of diarrhea and dysentery. Moreover, this study can be used as a tool for the isolation of pure antimicrobial from the plant and more works need to be done with the view of their use for *in-vivo* studies

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