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# ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF FRESH LATEX OF *EUPHORBIA THYMIFOLIA* LINN

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**ABSTRACT**
*Euphorbia thymifolia* Linn is small branched, pubescent, prostate annual herb with opposite oblong leaves, commonly known as laghududhika or choti-dudhi. Plant juice is employed in south India as cure for ring worms. The ethyl acetate extract and chloroform extract is reported to inhibit the growth of *E. coli* and *Shigella flexneri*. No scientific literature was found on medicinal properties of fresh latex. Hence the present work was designed to evaluate the traditional claims of *Euphorbia thymifolia* Linn. We have collected the fresh latex of plant by capillary action (1 kg of fresh sample gives 1.5ml of latex). The collected fresh latex was tested for antimicrobial activity by cylindrical cup plate method using standard cultures. Fresh latex showed maximum activity compare to diluted latex, dried latex, fresh juice and ethyl acetate, butanol, chloroform extracts of fresh plant. The antimicrobial activity of latex was found comparable with ciprofloxacin and fluconazole at tested concentration. The latex showed presence of tannins, resins, glycosides and sugars.
**KEY WORDS**: *Euphorbia thymifolia*, Latex, Antimicrobial activity, cylindrical cup plate method.

## INTRODUCTION

*Euphorbia thymifolia* Linn is small branched, pubescent, prostate annual herb with opposite oblong leaves, commonly known as laghududhika or choti-dudhi. It occurs throughout India in plains and low hills. Dried leaves and seeds are used as stimulant, astringent, anthelmintic, demulcent and laxative[1]. Root is given in amenorrhoea and gonorrhoea. Plant juice is employed in south India as cure for ring worms[1]. Juice of the powder plant is given with wine as remedy for bites of venomous reptiles and with ammonium chloride applied for cure of dandruff[1]. Charaka prescribed Dugdhika as an ingredient of vegetable soup for diarrhea, painful bleeding piles. According to Bhaavaprakasha, dugdhika is expectorant cures aggravated cough, skin disease, parasitic infection, promotes conception, possesses aphrodisiac and age- sustaining properties[2]. Plant yields a green essential oil with peculiar pungent odour and irritating taste with cymol, carvacrol, limonene and salicylic acid as constituents[3]. The ethyl acetate extract (0.45mg/ml) and chloroform extract(0.75mg/ml) is reported to inhibit the growth of E. coli and *Shigella flexneri* in vitro and ethyl acetate was active in vivo against *S. flexneri* [4]. The plant is also reported to have antioxidant, antiviral, anti lipid peroxidation and free radical scavenging activities[5]. Chemical constituents like hydrolysable tannin isomallotinic acid, crystalline alkaloid like principle similar to quercetrin, hexacosenol, euphorbol, epitaraxerol are reported[6-7]. Antimicrobial-resistant bacteria are the causes of numerous clinical problems worldwide. Infectious diseases caused by resistant microorganisms are responsible for increased health costs as well as high morbidity and mortality, particularly in developing countries. The increase in the prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs, and has necessitated the search for new antimicrobials from alternative or natural sources. One way to prevent antibiotic resistance is by using new compounds which are not based on the existing synthetic antimicrobial agents[8]. However, with the alarming increase of incidences of microbial resistance against many of these antimicrobial drugs, the need for newer, safer, and more effective antimicrobial drugs has become paramount[9]. Since the latex of the plant is traditionally used in the treatment of eye disorders and wounds in various parts of Kolhapur district and no literature was found on the latex. Hence in the present work authors have tried to evaluate the traditional claims on the latex of this plant along with some extracts for comparative studies. Further work on chemical investigation and other biological activities along with stability studies on the latex is going on.

## MATERIAL AND METHODS

The plant was located in nearby field around the college campus and was authenticated by Dr. Madhukar Bachulkar, Taxonomist and Principal Shri Vijaysinha Yadav Arts and Science College, Peth-Vadagaon, Dist. Kolhapur, Maharashtra. Herbarium (Voucher specimen no bvcop/cog-rgd-1) is deposited in the Pharmacognosy department of our college. Fresh plants were collected early in the morning and latex was collected by capillary action in glass screw cap bottle. Part of the latex was subjected for drying using vacuum dryer (Lab-Hosp) and remaining part was stored in the refrigerator for further use. Sample drug was collected in the due free morning and dried under shade for one week. Dried material was powdered in electrical blender (Bajaj) and coarse powder (# 40) was used for extraction. About 100g powder drug was macerated with 500ml of chloroform, butanol and ethyl acetate (Loba) and macerated for 72h at room temp in rotary orbital shaker (REMI, Mumbai) at 120rpm/min. Standard cultures were procured from NCIM Pune for antimicrobial activity and all other chemicals used were of analytical grade.

- **Extraction-** Powder (100g each) was macerated with 500ml of chloroform, butanol and ethyl acetate in macerating bottles and agitated at 120 rpm/min at room temperature for 72h in rotary orbital shaker. All the extracts were filtered and filtrate was evaporated in rotary film evaporator and dried in vacuum drier. The dried residue was weighed and % yield was calculated as given in **table 1.**
- **Fresh latex-** Fresh plants were collected early in the morning and stems were cut freshly and latex was collected (10ml) by capillary action in amber glass screw cap bottles. Part of the latex was subjected for vacuum drying and remaining fresh latex was used for antimicrobial activity. Another portion of the latex was kept in refrigerator (2-8$^0$C) for preservation and stability studies.
- **Fresh juice-** Fresh sample drug was collected and washed with distilled water and minced slightly and juice was obtained by squeezing the mass in fingers. The collected juice was filtered

through muslin cloth and used immediately for antimicrobial activity.

- **Phytochemical screening-** All the extracts obtained above were subjected for phytochemical screening using standard procedure[10-11]. The dried extracts (few mg) were dissolved in sufficient amount of respective solvents and tested for various constituents. The results are as per **table 2.**
- **Antimicrobial activity-** The activity was carried out by cup plate method as per standard procedure. All the standard cultures were freshly inoculated on the slants in respective growth media just before the start of experiment. Six standard bacterial cultures (*Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC-10031, *Psudomonas aeruginosa* ATCC-27853, *Salmonella typhi*) and three fungal strains (*Aspergillus niger* ATCC-16404, *Candida albicans* ATCC-10231, *Penicillium chrysogenus*) were tested for activity[12]. All the glass wares were sterilized by dry heat sterilization and culture media, normal saline distilled water was sterilized by moist heat sterilization. About 3ml of normal saline per plate was inoculated with each test organism using sterile inoculating loop just before the test and 25ml of sterile nutrient agar for bacterial strains and sabraud's dextrose media for fungal strains was poured in previously sterilized Petri plates at about $40^0$C. The inoculums were added with gentle swirling of the plates. Six wells in each plate were made with sterile borer (6mm dia.) and 100µl of sample, standard and control solutions were added with micropipette. The plates were kept on uniform platform for 3-4h to diffuse the samples uniformly and then bacterial cultures were incubated at $37^0$C in incubator for 24h and fungal cultures at room temperature for 48h. All the procedure was carried out in strict aseptic condition using horizontal laminar air flow. The zone of inhibition was measured with zone reader along with the diameter of borer as per **table-3.** Further MIC for fresh latex was determined by serial dilution method for all the tested organisms and results are given in **table-4**.

## RESULT AND DISCUSSION

Researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multiple drug resistant microbe strains[13]. There is an urgent need to discover new antimicrobial agents for human and veterinary therapeutic uses, as resistance to current drugs increases in severity and extent[14-17]. Plants are invaluable sources of pharmaceutical products[18]. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of disease[19]. Fresh latex has shown better antibacterial and antifungal activity with respect to all other test samples used. Fresh juice also shows activity with all tested organisms except *P. chrysozenous* and better than other test samples but less than fresh latex. Dried latex showed very weak activity compare to fresh latex. Diluted latex beyond 1:10 dilution failed to show antimicrobial activity. Among the extracts ETEA extract has shown better activity while ETBE and ETCE failed to show activity against *Salmonella typhi*. Fresh latex showed comparable antimicrobial activity with standard at tested concentration and its MIC ranges between 1:11 to 1:25 which exhibits very good and significant antimicrobial activity. The ETEA and ETBE extracts showed the presence of hexose and non reducing sugars along with glycosides, tannins, minerals and proteins. ETCE extract showed the presence of volatile oils, resins, alkaloids and steroids. The % yield was found maximum for butanol extract and least for chloroform. Many plant extracts have been reported for antimicrobial activity and correlated with the presence of tannins, phenolics and flavanoids such as *Camellia sinensis,*[20] *Rhizophora apiculata* bark,[21] blue and white flowering *Silybum marianum,*[22] roots of *Tecoma stans,*[23] punicalagin from the peel of *Punica granatum*, tannic acid from galls and prodelphinidin oligomers from the bark of *Elaeocarpus sylvestris* var. *ellipticus*[24]. This reveals that the antimicrobial activity of euphorbia thymifolia also may be due to the presence of tannins, phenolics or alkaloids.

From above results authors conclude that the fresh latex has very promising antimicrobial activity compare to all other extracts and fresh juice. It needs further investigation on chemical profile, stability, collection technique for latex so that a new potent antimicrobial agent will be obtained from natural source.

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**Table-1 Characterisation and % yield of extracts of *Euphorbia thymifolia***

| Sr.no. | Name of extract | Nature | Colour | % Yield |
|---|---|---|---|---|
| 1 | Chloroform | Sticky mass | Blakish | 2.450 |
| 2 | Butanol | Granular mass | Brownish | 11.27 |
| 3 | Ethyl acetate | Dry flakes | Blakish, shiny | 5.76 |

**Table-2 Phytochemical Screening of Extracts of *Euphorbia thymifolia***

| Constituents | Test | Extracts | | |
|---|---|---|---|---|
| | | ETCE | ETBE | ETEAE |
| Carbohydrates | Molisch's | - | + | + |
| Reducing sugar | Fehling's | - | + | + |
| | Benedict's | - | + | + |
| Monosaccharide | Barfoed's | - | + | + |
| Pentose sugars | Bial's Orcinol | - | - | - |
| Hexose sugars/Fructose | Selvinoff's | - | + | + |
| Galactose | Tollen's | - | + | + |
| Glucose/fructose | Cobalt-chloride | - | + | + |
| Non Reducing sugars | Hydrolyses test | - | + | + |
| Proteins | Biuret test | - | - | + |
| Steroids | Salkowaski | + | - | - |
| Cardiac glycosides | Legal | - | - | - |
| Anthraquinone glycosides | Borntrager's | - | + | + |
| Flavanoid glycosides | Shinoda | - | + | + |
| Alkaloids | Dragendorff's | + | - | - |
| Tannins | Ferric chloride | - | + | + |
| Organic acids | Calcium chloride | - | + | + |
| Vol.oils /resins | Filter paper | + | + | + |

+ indicates present and – indicates absent. ETCE: *Euphorbia thymifolia* Chloroform extract, ETBE: *Euphorbia thymifolia* Butanol extract, ETEAE: *Euphorbia thymifolia* Ethyl acetate extract.

**Table-3 Zone of inhibition of *Euphorbia thymifolia* extracts and latex**

| Test samples | Conc Used | Zone of inhibition * in mm | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. coli* | *B. sub.* | *P. aeur.* | *K. pnum.* | *S. aure.* | *S. typ.* | *A. nig.* | *C. alb.* | *P. chr.* |
| ETCE | 50mg/ml | - | 11 | 12 | 15 | 15 | - | 22 | 21 | - |
| ETBE | 50mg/ml | 18 | 20 | 18 | 24 | 20 | - | 19 | 18 | 25 |
| ETEAE | 50mg/ml | 19 | 22 | 22 | 24 | 23 | 11 | 20 | 20 | 20 |
| Fresh latex | 100µl | 33 | 25 | 24 | 24 | 25 | 23 | 24 | 25 | 26 |
| Dried latex | 10mg/ml | 11 | 10 | 11 | 10 | 09 | - | 11 | 12 | 10 |
| Diluted latex | 1:10 | 23 | 17 | 15 | 10 | 11 | 12 | 16 | 17 | 18 |
| | 1:50 | - | - | - | - | - | - | - | - | - |
| | 1:100 | - | - | - | - | - | - | - | - | - |
| Fresh juice | 100µl | 22 | 21 | 20 | 22 | 18 | 22 | 21 | 23 | - |
| Ciprofloxacin | 10µg/ml | 23 | 21 | 21 | 24 | 22 | 23 | NT | NT | NT |
| Fluconazole | 10µg/ml | NT | NT | NT | NT | NT | NT | 18 | 17 | 18 |
| Standard control | 100µl | - | - | - | - | - | - | - | - | - |
| Sample control | 100µl | - | - | - | - | - | - | - | - | - |

* Average of three replicate readings including external borer diameter

ETCE: *Euphorbia thymifolia* chloroform extract, ETBE: *Euphorbia thymifolia* butanol extract, ETEAE: *Euphorbia thymifolia* ethyl acetate extract. NT: not tested, - : no action

**Table-4 MIC determination of fresh latex of *Euphorbia thymifolia***

| Test organism | MIC of fresh latex |
|---|---|
| *Escherichia coli* ATCC 8739 | 1:25 |
| *Bacillus subtilis* ATCC 6633 | 1:12 |
| *Psudomonas aeruginosa* ATCC-27853 | 1:15 |
| *Klebsiella pneumoniae* ATCC-10031 | 1:15 |
| *Staphylococcus aureus* ATCC 25923 | 1:13 |
| *Salmonella typhi* | 1:11 |
| *Aspergillus niger* ATCC-16404 | 1:16 |
| *Candida albicans* ATCC-10231 | 1:13 |
| *Penicilleum chrysozenous* | 1:12 |

Results after respective dilutions of fresh latex still showing inhibitions

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