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Study the effect of UV light on the antimicrobial activity of *Euphorbia hirta* leaf extract

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Abstract
The aim of the present study was to investigate the phytochemical screening and antimicrobial activities of our selected medicinal plant *Euphorbia hirta* leaf extracts in addition to these study we also evaluate the of UV light radiation on the anti microbial activity of leaf extract of selected medicinal plant. Phytochemical screening revealed the presence of various active phytoconstituents in the extracts of aerial part of *Euphorbia hirta due these phytochemicals* our plant show significantly antimicrobial activity. The antimicrobial activity of the extracts generally reduces significantly after exposure to the UV radiations.

Keywords: *Euphorbia hirta*, antimicrobial activity, anti fungal activity, UV light

Introduction
Medicinal plants are gifts of nature which is store house of remedies to cure limitless number of diseases of human beings. The plant kingdom represents an enormous reservoir of biologically active compounds with different chemical structures and protective or preventive properties these biologically active compounds are called as phytochemicals. These phytochemicals are generally secondary metabolites which are present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. Biologically active compounds are widespread from plant sources have always been of great interest to researcher working on infectious diseases. Over the past decade there has been an explosion of interest among the researcher in the antimicrobial, particularly antibacterial and antifungal, activity of natural products the abundance of plants on the earth's surface has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants, as potential source of new antimicrobial agents.
Euphorbia hirta is a medicinal, rhizomatous herb belonging to Euphorbiaceae family generally found in southern Western Ghats of India and northern east coast of Tamil Nadu. In African countries the extract of this plant are commonly used in the treatment of asthma and respiratory tract inflammations. It is also used for coughs, chronic bronchitis, and other pulmonary disorders in Malagasy. The plant is also widely used in Angola against diarrhea and dysentery, especially amebic dysentery. In Nigeria, extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore, and promoting wound healing.
The use of plant extracts and phytochemicals with known antimicrobial properties, can be of great significance in therapeutic treatments. This Herbal and natural products have been used in herbal medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, this coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. But the majour challenges facingin herbal medicine industries are the overall quality safety and and efficiency of the herbal medicines. There are so many reports have been published to report that the efficiency of the herbal medicine is significantly decreases due to exposure of sunlight. With this respect in current study we study the effect of UV light on the antimicrobial activity of leaf extract of our selected plant euphorbia herita.

Materials and Methods
Plant Collection and Authentication
The leaves of *Euphorbia hirta* was collected from the different part of dehradun and authenticated by Botany department of FRI, Dehradun. The leaves of selected plant was washed thoroughly 2-3 times with running water and once with sterile distilled water.

Preparation of extract
Shade-dried plant leaves of euphorbia herita was converted in to small pieces by using mortar and pestle grinded into powdered form The powdered plant material was udergo to sequential solvent extraction by using soxhlet extraction method. The extraction was done with different solvents in their increasing order polarity such as hexane, chloroform and ethanol. The isolated extract was evaporated using rotary evaporator and the percentage yield was thus recorded. Dried extracts were stored in airtight containers for further studies. Concentrated extracts were subjected to various chemical tests in order to detect the various phytoconstituents.

Phytochemical screening
The concentrated extracts of selected plant was subjected to different chemical tests for the detection of different phytoconstituents using standard methods [19, 20].

(i) Test for saponins
Crude extract when mixed with 5ml distilled water in a test tube then it was shaken briskly. The formation of stable foam which indicate the presence of saponins.

(ii) Test for flavonoids
Crude extract when mixed with 10ml distilled water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate solution then added 1ml concentrated sulphuric acid. Indication of yellow color shows the presence of flavanoids.

(iii) Test for steroids
The crude extract of selected plant was dissolved in 0.5mL dichloromethane to prepare a dilute solution and then 0.5 mL of acetic anhydride was added followed by four drops of concentrated sulphuric acid. A blue-green colouration indicated the presence of steroids.

(iv) Test for tannins
Curde extract of plant was mixed with small amount of water and heated on water bath. The mixture was filtered and ferric chloride was added drop by drop to the filtrate. A dark green appear which indicates the presence of tannins.

(v) Test for Alkaloids
Curde extract was dissolved with 2ml of 1% HCl and heated gently. Wagners and Mayers reagents were added to the mixture. Turbidity of the resulting precipitate was taken as confirmation for the presence of alkaloids.

(vi) Test for carbohydrate
Both Felhing A and Felhing B solution were mixed in equal volume. These reagent are added in crude extract and smoothly boiled. A brick red precipitate is appeared at the bottom of the test tube and indicate the presence of reducing sugar.

Microbial samples
The human becteria such as *Streptococcus mutans, Clostridium absonum, and Escherichia coli* were obtained from department of biotechnology of JS university and were maintained in Nutrient agar at 4 ºC for experiment studies. The different fungus strains such as *Arthogrophis cuboida, Aspergillius fumigates* and *Aspergillius nigar* were isolated from potato dextrose agar.

Screening for Antibacterial Activity
Assay of anti-bacterial activity of leaf of *Euphorbia hirta* was done by Disc Diffusion method. In this method 30 ml of sterilized Mueller Hinton Agar was poured into sterile petri plates, after solidification, 130 µl of bacterial culture poured on the plates and the culture was spread on plates using spreader. Then, the Whatmans filter paper discs (5mm in diameter) were kept over the agar plates using sterile forceps at various concentrations. Concentrated solvent was used as negative control. The anti-bacterial assay plates were kept incubator, where all the plates were incubated at 37ºc for 24hours. The diameter of inhibition zone was noted down.

Screening for Antifungal Activity
Assay of anti-fungal activity of leaf extract of *Euphorbia hirta* was done by Disc Diffusion method. In this method 30 ml of sterilized Mueller Hinton Agar was poured into sterile petri plates, after solidification, 130µl of fungus culture poured on the plates and the culture was spread on plates using spreader. Then, the What Mans filter paper discs (5mm in diameter) were kept over the agar plates using sterile forceps at various concentrations. Concentrated solvent was used as negative control. The anti-fungus assay plates were kept incubator, where all the plates were incubated at 36ºc for 24hours. The diameter of inhibition zone was noted down.

Photo irradiation
The isolated leaf extract of *Ephorbia herita* was subjected to the photoirradiation with 254 nm UV light and for this purpose first we prepare the solution of our extract in ethanol chloroform and hexane after that we irradiated with UV light of 254 nm for limited time period for 1 hr. Antimicrobial activity of samples against the microorganism was evaluated after the period of irradiation.

Result and Discussion
In the present study, different leaf extract of *Ephorbia herita* were subjected to qualitative phytochemical analysis to explore its anti microbial activity for its medicinal applications.
The percentage yields of leaf extracts with different solvent and the phytochemical constituents of the plants are shown in table 1 and 2 respectively. The highest yield of leaves extract was found when extraction was done with ethanol and the lowest in case of hexane. This is most probably due to change in the polarity of solvents.

Table 1: The Yield of extract with different solvent (%)

Plant Sample	Ethanol Extract (%)	Chloroform Extract (%)	Hexane Extract (%)
Leaves	34	21	12

Table 2: Preliminary phytochemical analysis of different leaves extract of *Calotropis gigantean*

Phytochemical constituents	Ethanol	Chloroform	Hexane
Alkaloids	+	+	_
Flavonoides	+	+	+
Terpenoids	+	+	_
Tannins	-	+	_
Saponins	-	_	_
Carbohydrates	-	+	_

+ = indicates presence of phytochemicals
- = indicates absence of phytochemicals.

The result of the preliminary phytochemical screeing of different leaves extract of *Euphorbia herita* shows in table 2. The present study reveals that the phytochemical screening and qualitative estimation of leaves extract of *Euphorbia herita* showed the presence of alkaloid, flavanoid, tannin, terpenoid and carbohyrate in choroform. In ethanolic extract of leaves alkaloid, flavanoid, sponins, carbohydrate and terpenoids are present.In the hexane extract of leaves only flavonoid is presnt.

Antibacterial activity

Result of the antibacterial activity of the isolated leaf extract of our selected plant was shown in table 3. The dried leaves extract of *euphorbia extract* shown to posse's antibacterial activity. The antibacterial activity of ethanol, chloroform and hexane of extract of leaves of *euphorbia extract* were inspected against the selected experiment pathogens such as *Streptococcus mutans, Clostridium absonum, and Escherichia coli* by disc diffusion method. The ethanolic leaf extract of *euphorbia herita* showed the maximum zone of inhibition against in *Clostridium* (32 mm) which is gram positive bacteria and cause several diseases such as food poisoning, pneumonia and brain abscess. The hexane extract of leaves extract of *euphorbia herita* showed minimum zone of inhibition against *Escherichia coli* (12 mm).

Table 3: The Zone of inhibition (mm) of different extracts against the tested bacteria

Microorganisms	Leaves Extract (zone of inhibition in mm)		
	Ethanol	Chloroform	Hexane
Clostridium absonum	32	27	24
E-coli	28	21	12
Streptococcus mutans	22	17	13

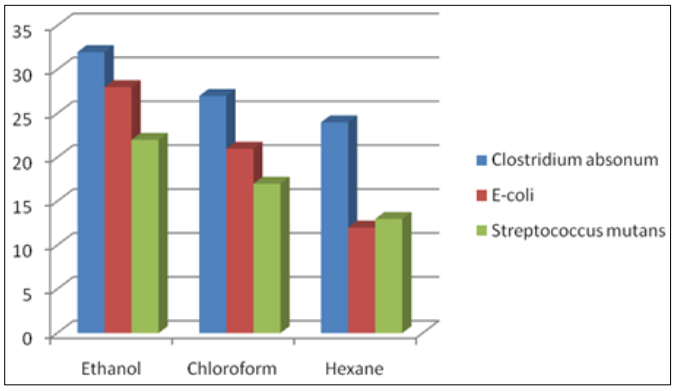

Fig 1: *Clostridium absonum* E-coli *Streptococcus mutans*

Antifungal activity

Result of the antifungal activity of the isolated extract by using different solvent (ethanol, chloroform, and hexane) was showed in table 4. The antifungal activity of ethanol, chloroform and hexane of extract of dried leaves of *euphorbia herita* were inspected against the selected experimental pathogens such as *Arthrographis cuboid, Aspergillus fumigates* and *Aspergillus niger* by disc diffusion method. The ethanol leaf extract of *Calotropis gigantea* showed the maximum zone of inhibition against in *Arthogrophis Cuboida* (39 mm). The hexane extract of leaves extract of *Calotropis gigantea* showed minimum zone of inhibition againest *Aspergillius nigar* (10 mm). Minimum Inhibitory Concentration (MIC) of *Calotropis gigantean* was also determined and the result was shown in table-5

Table 4: Antifungal activity of leaves of *Euphorbia herita*

Microorganisms	Leaves Extract (zone of inhibition in mm)		
	Ethanol	Chloroform	Hexane
Arthogrophis Cuboida	39	31	23
Aspergillius fumigates	29	26	18
Aspergillius nigar	25	20	10

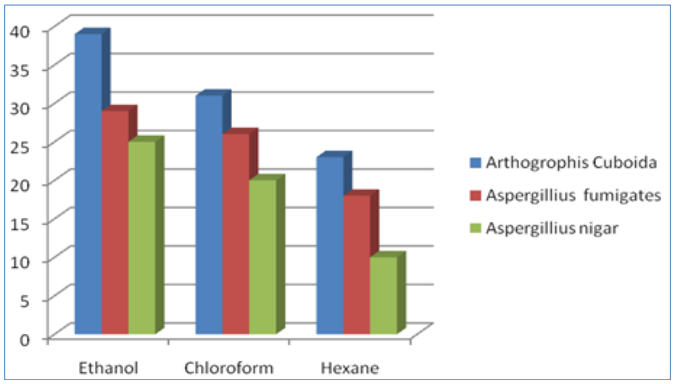

Fig 2: *Arthogrophis cuboids Aspergillius fumigates* Aspergillus Niger

The evaluation of the antimicrobial activity of the irradiated samples (ethanolic sample) revealed a major loss of activity (Table 1). The loss in activity of the UV irradiated samples proposes that, photochemical degradation of the active compounds has resulted in structural modifications of functional group (s) required by the compounds for the antimicrobial activity.

Table 5: Effect of UV light on antibacterial activity of ethanolic leaf extract of *Calotropis gigantean*

Microorganisms	Zone of inhibition (Non-treated)	Zone of inhibition (treated)
Clostridium absonum	32 mm	18 mm
E-coli	28 mm	12mm
Streptococcus mutans	22mm	10 mm

Table 6: Effect of UV light on antifungal activity of ethanolic leaf lextract of *Calotropis gigantean*

Microorganism	Zone of inhibition (Non-treated)	Zone of inhibition (treated)
Arthogrophis Cuboida	39 mm	20mm
Aspergillius fumigates	29 mm	17mm
Aspergillius nigar	25mm	12 mm

Conclusion

The finding of our study clearly indicate that the Euphorbia herita may be used as potential antimicrobial agent but in this study we also established that the over exposure of UV light may gradually decreases the antimicrobial capacity of this plant hence form this study we established that herbal medicine may be used as a potential antimicrobial agent but its protected from the direct exposure to light.

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