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Antimicrobial studies on flowers of *Euphorbia Milii*

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ABSTRACT

Euphorbia milii (Euphorbiaceae) is an ornamental plant plays a role in folk medicine. The Chinese use it as a cure for cancer, and some Brazilians believe that it can cure warts. The plant material of Euphorbia milii was collected from Kadiyapulanka, Rajahmundry, East Godavari District, A.P, India, in September 2014.The Antimicrobial activity studies of hexane, ethyl acetate, acetone, methanol and water extracts of flowers of Euphorbia milii (Euphorbia) were performed on gram positive organisms, Bacillus subtilis, Staphylococcus aureus, and gram negative organisms Escherichia coli and Proteus vulgaris by using cup plate method. The hexane, acetone and methanol extracts in the concentration of 5µg/ml, have shown considerable inhibition zone on Staphylococcus aureus& Bacillus subtilis as compared to other extracts. The hexane, acetone and methanol extracts in the concentration of 5µg/ml, have shown considerable inhibition zone on Escherichia coli & Proteus vulgaris as compared to ethyl acetate and water extracts which was compared with the inhibition zone produced by standard amikacin sulphate (1µg/ml.The estimation of phenolic compounds of hexane, ethyl acetate, acetone, methanol and water extracts of flowers of the plant Euphorbia milii (Euphorbia) were determined with the Folinciocalteu's reagent (FCR) by UV-Visible Spectrophotometric method. The concentrations of the extracts were selected as 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml, after performing the assay the results indicating that hexane and acetone extracts contain more amounts of phenolic compounds than other extracts. These studies, indicates that the plant Euphorbia milii (Euphorbia) contains phenolic compounds which can act against selective gram positive and gram negative organisms.

Keywords: *Euphorbia milii*, anti-microbial, FC-Reagent, UV-Visible Spectroscopy.

INTRODUCTION

The author thought to discuss some of the important features of flavonoid compounds as the present phytochemical work carried out was mainly concern with the flavonoid compounds. Flavonoids are yellow pigments [1], which occur in plant kingdom either in the free state or as glycosides or associated with tannins. These are also known as the anthoxanthins. Chemically, the flavones are hydroxylated derivative of flavone (2-phenyl-4-chromone), which are partially alkylated. In most of the flavones, positions 5 and 7 are hydroxylated and also one or more of positions 3, 4, 5 are also hydroxylated. Further, positions 3^1 and 5^1 are often methylated whereas positions 5, 7 and 4^1 are usually unmethylated.

Euphorbiaceae [2] is a large family of flowering plants with about 300 genera and 7,500 species most *spurges* are herbs, but some, especially in the tropics, are shrubs or trees. Some are succulent and resemble cacti because of convergent evolution. Laypersons may refer to euphorbias having a growth form and succulence similar to cacti as being "cactuses". The plant *Euphorbia milii* of Euphorbiaceae family was selected, Flavonoids were extracted by Soxhlet extraction. The anti microbial properties of the plant *Euphorbia milii* studied by cup plate method. The estimation of phenolic compounds of hexane, ethyl acetate, acetone, methanol and water extracts of flowers of the plant *Euphorbia milii*were determined with the folinciocalteu's reagent (FCR) using UV-Visible Spectrophotometric method.

Taxonomical Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Euphorbiaceae
Genus	Euphorbia
Species	*E.Milii*
Origin	Madagascar
Flowering	Yes
Fragrant	Slightly
Growing case	No extra care requires
Temperature	30-40^{o}C
Vernacular names	Crown of thorns, Christ plant. Christ thorn

MATERIALS AND METHODS

Plant material:
The plant material of *Euphorbia milii* was collected from Kadiyapulanka, near to Rajahmundry, East Godavari District, A.P, India, in September 2014 and identity was confirmed by Botany Department, Satyanarayana Nursery, Kadiyapulanka, East Godavari, Andhra Pradesh. The specimen was kept in the herbarium of the Pharmacognosy & Phytochemstry Division of VJ's college of Pharmacy, Diwancheruvu, Rajahmundry.

Instruments: UV Spectrophotometer- Double Beam UV Spectrophotometer UV-2202(Systonics), Soxhslet extractor, Simple distillation apparatus & Auto clave (Kshitij Innovations), laminar air flow (Kirloskar-electrodyne), Hot air oven & Incubator (KEMI- kadavil electro Mechanical industries- KOMA: 3), Electronic balance (Eagle), Physical balance (Kshitij Innovations) and glass ware (Borosil) -Sterilized Petri dishes, pipettes, boiling tubes, beakers, Sterilized test tubes, Sterile 6mm cork borer, Sterile inoculation loop, Sterilized fine pointed forceps, Tuberculin syringes.

Chemicals: All Chemicals used were analytical grade and purchased from Merk Chemicals Pvt., Ltd. Mumbai, India. Hexane, Ethyl acetate, Acetone, Methanol, FC reagent, Gallic acid Standard, Sodium carbonate, Amikasinsulphate (MIKACIN)..

Culture media: Nutrient agar medium (Composition: Peptone-, Agar-, Beef Extract-, Nacl-, Distilled Water-1000ml), 8 to 10 hours old growth cultures in nutrient broth from Jagruty Labs, Rajahmundry.

Antimicrobial activity of Euphorbia milli was identified by following methods:
The antibacterial activity of extracts collected from the flowers of the plant *Euphorbia milii (*Euphorbiaceae) [3-9]were studied comparatively with that of standard antibiotic amikacinsulphate (MIKACIN-1mg/ml) by cup plate method using gram positive organisms Bacillus subtilis, Staphylococcus aureus and gram negative organisms namely Escherichia coli and proteus vulgaris.

Preparation of Extracts: Flowers of *Euphorbia milii* (Euphorbiacae) were shade dried for 5days and powdered. The plant components were extracted in Soxhlet apparatus with hexane, ethyl acetate, acetone, methanol and water respectively. Extracts thus obtained from the flowers of the plant by soxhlet extraction were used for the estimation of anti-microbial activity and estimation of total Phenolic compounds.

Preparation of Media:
The organism used in the present study for evaluating antibacterial activity of test compounds were obtained from the laboratory stock .On the day of testing, the organisms were sub cultured in to sterile nutrient broth . After incubating the same for three hours, the growth thus obtained was used inoculums for the test.

Sterilization of Media and Glassware:
The media used in the present study, nutrient agar and nutrient broth, were sterilized in the conical flasks of suitable capacity by autoclaving at 15 lbs pressure for about 20 minutes. The cork borer, petridish, test tubes and pipettes were sterilized in hot air oven at 160^0c for one hour.

Preparation of Solutions:
Standard (Amikacin): 100 mg of amikacin was dissolved in 100 ml of sterile water to get a concentration of 1mg/ml. And it is further diluted with water to get the concentration of 1ug/ml.

Test compound (Extract): 500 mg of each test compound was dissolved in 10 ml of hexane, ethyl acetate, acetone, methanol, water in serial and suitably labeled sterile test tubes, thus given a final concentration of 50mg/ml.

Method of Testing:
Cup Plate Method:
This method depends on the diffusion of an antibiotic form activity through the solidified agar layer in a petridish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity. A previously liquefied medium was inoculated appropriated to the assay with the requisite quantity of the suspension of the microorganisms between 40-50^0c and the inoculated medium was poured in to petridish to give a depth of 3-4mm.It was ensured that the layers of medium were uniform in thickness by placing the dishes on a leveled surface .The dishes thus prepared was stored in a manner so as to ensure that no significant growth or death of the test organisms occurs, before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, two cups of each 6 mm diameter was punched and scooped out of the set agar in each petridish (three cups were for the particular compound and standards).Using injection the standards blank and the test sample solutions (0.1 ml) of known concentration were fed in to the borer cups .The order of the solutions was as follows.

- Standard (Amikasinsulphate).
- Blank solution.
- Test compound (Extract).

The dishes were left standing for one to four hours at room temperature at a period of pre incubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for 24 hrs for 37^0c. The zone of inhibition developed, if any, was then accurately recorded. Each zone of inhibition recorded was average of three measurements. Results and statistical analysis was presented in tables.

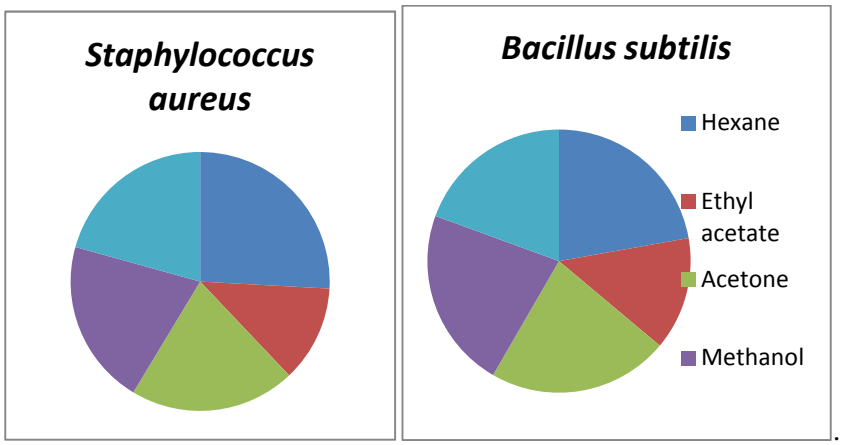
Estimation of Phenolic compounds by using spectrophotometry:
Assay Method:
The phenolic content in hexane, ethyl acetate, acetone, methanol and water extracts were determined with the Folinciocalteu's reagent (FCR). 1ml of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml concentrations of hexane, ethyl acetate, acetone, methanol and water extracts were mixed with 0.1ml (FCR). After 5mins 2ml of 20% sodium carbonate solution was added the final volume of the tubes were made up to 10ml with distilled water and allowed to stand for 10mins at room temperature. Absorbance of sample was measured against the blank at 720nm using a UV-Visible Spectrophotometer. The results were compared with Gallic acid standard results.The F–C assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic / phosphotungstic acid complexes, which are determined spectroscopically at 760 nm. Although the electron transfer reaction is not specific for phenolic compounds.

RESULTS AND DISCUSSION

Table 1: Inhibition Zones produced by sample on both gram+ve and gram-ve organisms

S.No	Name of the Bacteria	Sample (50mg/ml)	Inhibition zone (mm)	Standard (1mg/ml)	Inhibition zone (mm)
1	*Staphylococcus aureus*	Hexane	15	Amikacin sulphate	25
2	*Staphylococcus aureus*	Ethyl acetate	7	Amikacin sulphate	24
3	*Staphylococcus aureus*	Acetone	14	Amikacin sulphate	25
4	*Staphylococcus aureus*	Methanol	12	Amikacin sulphate	26
5	*Staphylococcus aureus*	Water	5	Amikacin sulphate	26
6	*Bacillus subtilis*	Hexane	16	Amikacin sulphate	28
7	*Bacillus subtilis*	Ethyl acetate	10	Amikacin sulphate	28
8	*Bacillus subtilis*	Acetone	16	Amikacin sulphate	27
9	*Bacillus subtilis*	Methanol	12	Amikacin sulphate	28
10	*Bacillus subtilis*	Water	5	Amikacin sulphate	29
11	*Escherichia coli*	Hexane	16	Amikacin sulphate	30
12	*Escherichia coli*	Ethyl acetate	8	Amikacin sulphate	31
13	*Escherichia coli*	Acetone	18	Amikacin sulphate	30
14	*Escherichia coli*	Methanol	11	Amikacin sulphate	31
15	*Escherichia coli*	Water	5	Amikacin sulphate	33
16	*Proteus vulgaris*	Hexane	15	Amikacin sulphate	31
17	*Proteus vulgaris*	Ethyl acetate	10	Amikacin sulphate	33
18	*Proteus vulgaris*	Acetone	15	Amikacin sulphate	31
19	*Proteus vulgaris*	Methanol	14	Amikacin sulphate	32
20	*Proteus vulgaris*	Water	6	Amikacin sulphate	32

Graph 1: Inhibition zone obtained for Gram Positive organisms



Graph 2: Inhibition zone obtained for Gram Negative organisms

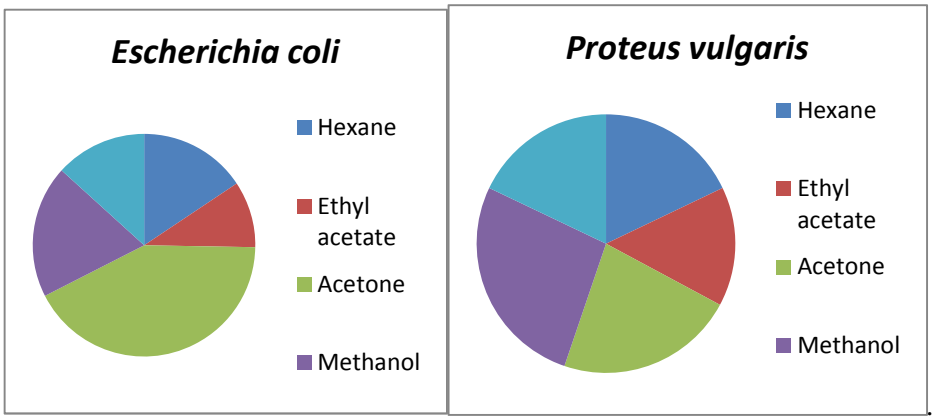


Fig 1: Inhibition Zone produced by Hexane extract

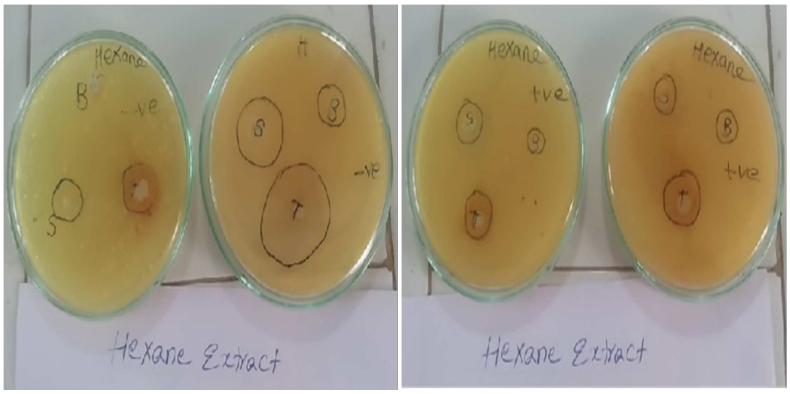


Figure 2: Inhibition Zone produced by Ethyl acetate extract

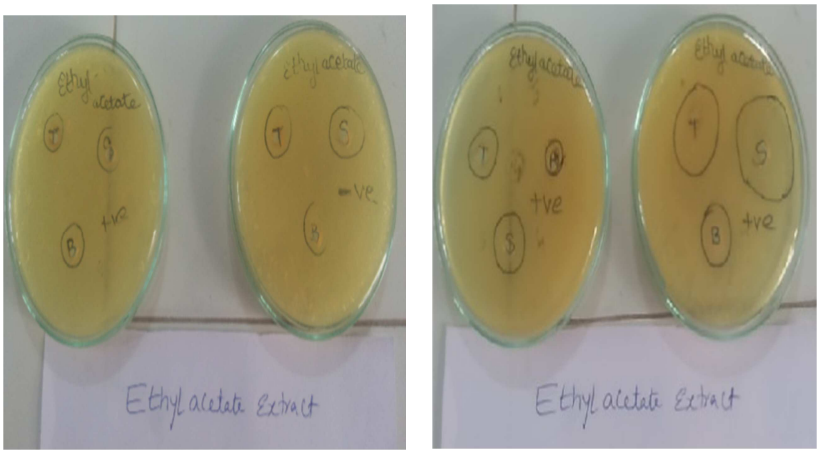


Fig 3: Inhibition Zone produced by Acetone extract

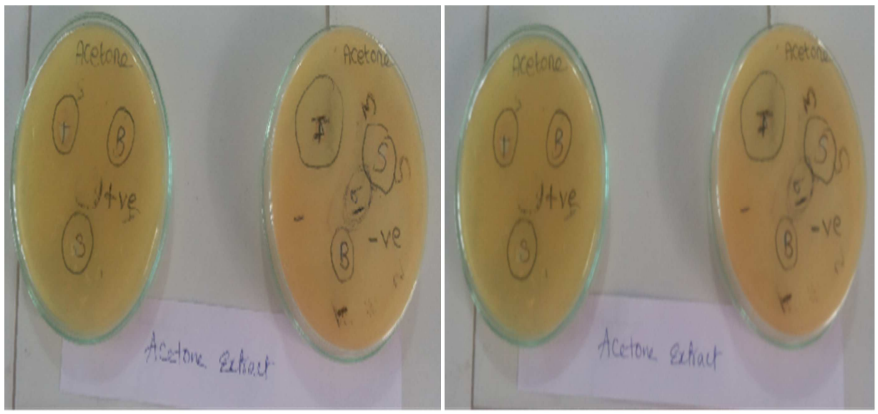
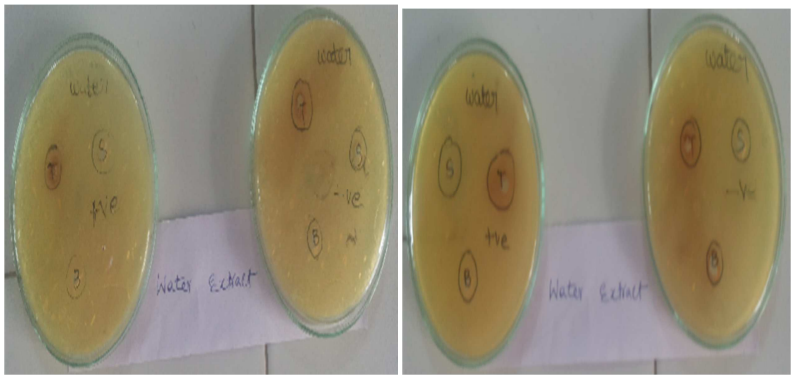


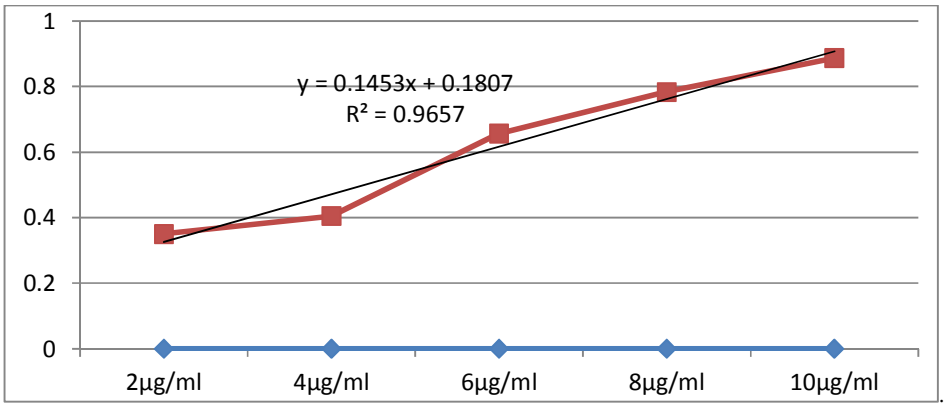
Fig 4: Inhibition Zone produced by Methanol extract

Fig 5: Inhibition Zone produced by water extract



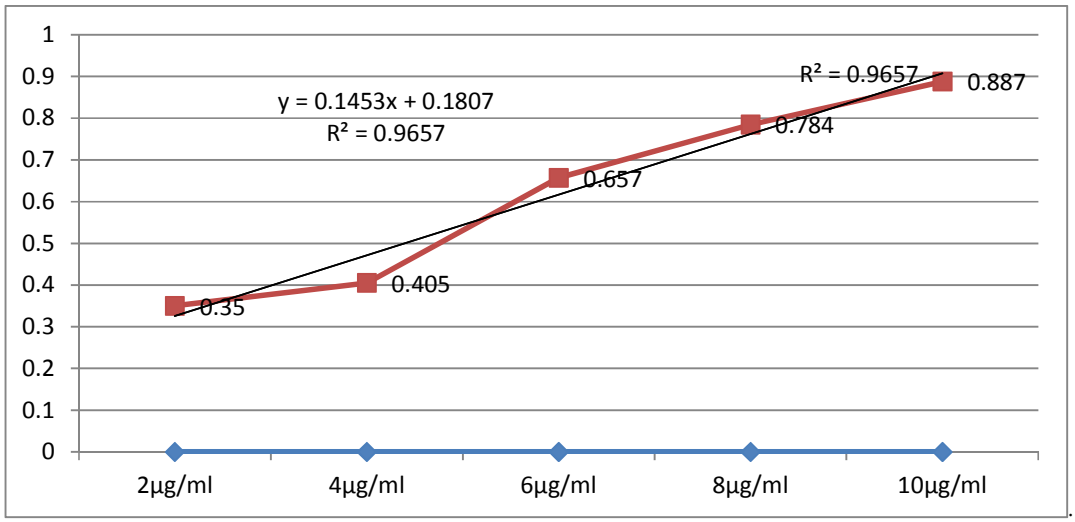
Hexane extract:

S.No	Concentration	Absorbance
1	2µg/ml	1.9
2	4µg/ml	1.93
3	6µg/ml	1.574
4	8µg/ml	1.488
5	10µg/ml	1.996



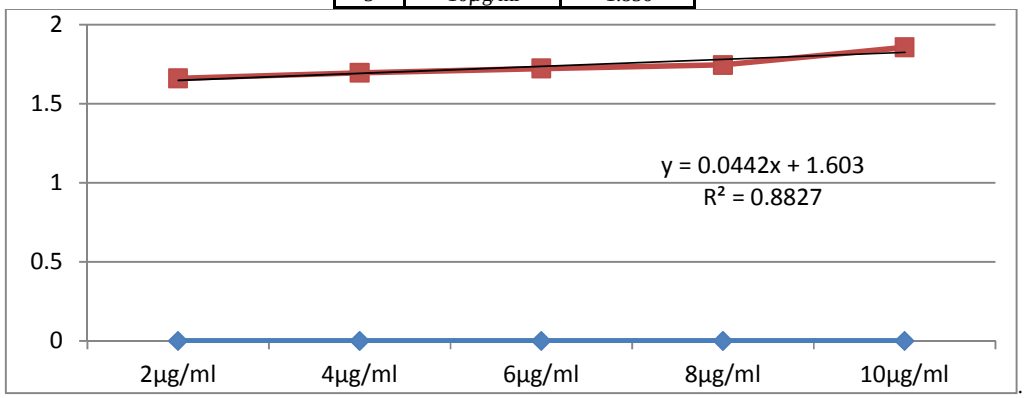
Ethyl acetate extract:

S.No	Concentration	Absorbance
1	2µg/ml	0.35
2	4µg/ml	0.405
3	6µg/ml	0.657
4	8µg/ml	0.784
5	10µg/ml	0.887



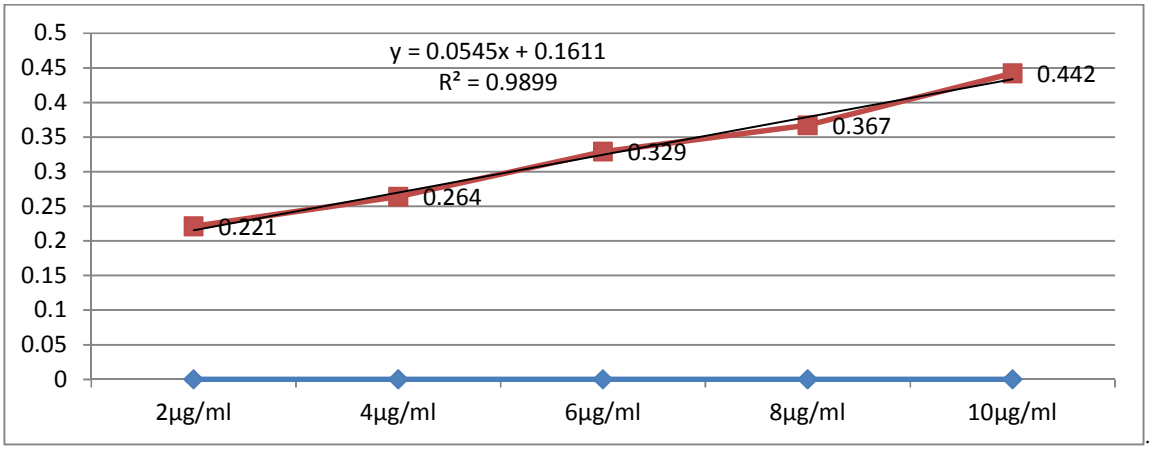
Acetone extract:

S.No	Concentration	Absorbance
1	2µg/ml	1.66
2	4µg/ml	1.695
3	6µg/ml	1.722
4	8µg/ml	1.745
5	10µg/ml	1.856



Methanol extract:

S.No	Concentration	Absorbance
1	2µg/ml	0.221
2	4µg/ml	0.264
3	6µg/ml	0.329
4	8µg/ml	0.367
5	10µg/ml	0.442



Spectrophotometric identification of extracts obtained from *Euphorbia milii*:

UV-Visible Spectrum of hexane, ethyl acetate, methanol, water extracts of ***Euphorbia milii*** were shown in the figure 01 – 04 and the values of absorbance at different wave lengths were as follows.

Table 2: Absorbance & Wave lengths of the extracts obtained by soxhlet extraction

S.No	Name of the extract	Absorbance	Wave length (nm)
1	Hexane	4.001 3.867	289.6 267.2
2	Ethyl acetate	4.010 2.155	256.1 317.6
3	Methanol	2.463 2.348 2.198 1.694 0.776	231.2 276.8 329.6 264.8 207.2
4	Water	2.755 2.226 0.042	233.6 329.6 387.2

Antimicrobial activity:

The Antimicrobial activity studies of hexane, ethyl acetate, acetone, methanol and water extracts of flowers of the plant *Euphorbia milii* (Euphorbia) were performed on gram positive organisms Bacillus subtilis, Staphylococcus aureus, and gram negative organisms namely Escherichia coli and Proteus vulgarisby using cup plate method. The results thus obtained by this method were indicating that the extracts have shown the activity on both gram positive and gram negative organisms as shown above.

The hexane, acetone and methanol extracts in the concentration of 5µg/ml, have shown considerable inhibition zone on gram positive bacteria Staphylococcus aureus & Bacillussubtilis as compared to ethyl acetate and water extracts which was compared with the inhibition zone produced by standard amikacinsulphate (1µg/ml).

The hexane, acetone and methanol extracts in the concentration of 5µg/ml, have shown considerable inhibition zone on gram negative bacteria Escherichia coli &Proteus vulgaris as compared to ethyl acetate and water extracts which was compared with the inhibition zone produced by standard amikacin sulphate (1µg/ml). It indicates that the flowers of the plant contain the chemical constituents which can act against the micro organism [10-11].

Estimation of Phenolic compounds by using spectrophotometry:

The estimation of phenolic compounds of hexane, ethyl acetate, acetone, methanol and water extracts of flowers of the plant *Euphorbia milii* (Euphorbia) were determined with the folinciocalteu's reagent (FCR). The concentrations of the extracts wereselected as 2µg/ml, 4µg/ml, 6µgml, 8µg/ml and 10µg/ml, after performing the assay the results

indicating that hexane extract and acetone extracts contain more amounts of phenolic compounds as that of ethyl acetate, methanol and water extracts.

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