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Research Article

To investigate phytochemical parameter, Pharmacognostical evaluation, pharmacological (anti-bacterial) activity of the extract of *Euphorbia pulcherrima*

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Abstract

Introduction: *Euphorbia pulcherrima* is member of Euphorbiaceae family of medicinal plants, and it is used to cure variety of ailments which includes toothache, vomiting, fractures, severe bleeding, bruising, and hypermenorrhoea. *Euphorbia pulcherrima* has been found to possess a variety of chemicals, including alkaloids, steroids, terpenoids, flavonoids and reducing sugars. The review analyses its pharmacological profile, chemical component, and phytochemistry, with an emphasis on antioxidant activity, antimicrobial activity, Osteoclast activity, analgesic activity, anti-inflammatory activity, sedative activity, muscle relaxant activity, anticonvulsant activity and toxicological profile, in order to better assess the plants medicinal potential. Traditionally the latex has been employed as an anti-vomiting and toothache treatment. Aches and pains have been treated with leaf poultices. In traditional Chinese medicine it has been used it to treat fractures, severe bleeding, bruising, and hypermenorrhoea.

Methods: Due to extensive research on the pharmacological effects and active compounds of *Euphorbia pulcherrima*, it has a great deal of potential in modern medicine. Phyto, physicochemical standardization of dried, matured root of *Euphorbia pulcherrima* has been carried out in the present study. The study includes organoleptic characters along with estimation of its physicochemical parameters such as loss on drying, ash values, extractability in water and methanol. Preliminary phytochemical screening and anti-bacterial assay has been conducted.

Conclusion: As there is not enough evidence for detailed physicochemical and phytochemical evaluation on root of *Euphorbia pulcherrima* is reported. The present study reveals that the selected plant extract produces antimicrobial activity with dose dependent manner. However, it needs further evaluation in clinical settings before consideration for the treatment of different diseases.

Keywords: *Euphorbia pulcherrima*, Phytochemicals, pharmacological activity, Physicochemical.

INTRODUCTION

The primary source of numerous classes of secondary metabolites is medicinal plants[1]. There is a connection between plants' medicinal efficacy and the presence of natural chemicals. Several bioactive natural compounds have been identified and are important components of contemporary medical system[1]. One of the most significant sources of medicines comes from plants. Plant-derived chemicals, or phytochemicals, are gaining popularity as natural substitutes for manufactured chemicals. All traditional medical systems are based on the use of plant extracts to treat a variety of ailments. The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries[2]. Today's advances in technology in chemical, pharmacological, and microbiological research have made it possible to effectively screen higher plants for active chemicals[2]. Numerous plants are being studied for the development of novel drugs since medications made from plants are more potent yet less toxic.

E. pulcherrima is a perennial herb that is well known for treating a range of diseases. It is found throughout the world's tropical and subtropical regions[3].*Euphorbia pulcherrima* (EP) belongs to family: Euphorbiaceae and genus: Euphorbia (English name: Poinsettia [Christmas star], Nepali name: Lalupate, Hindi name: Lalpatta).Popular Christmas plants like *Euphorbia pulcherrima* are planted for their scarlet leafy bracts .It is a flower with Mexican ancestry that is endemic to the Pacific coast of the United States, some regions of central and southern Mexico, including the Mexican Pacific coast, and a few locations in Guatemala. It is commonly referred to as a poinsettia. Additionally, it is also readily accessible throughout Nepal, particularly in the hilly area[4].

MATERIALS AND METHODS

Collection and identification of the plant materials

The roots of *Euphorbia pulcherrima* was collected from 6th mile Tadong, Gangtok Sikkim Himalayan region in month of September 2022. The plant was identified by Botanical Survey of India, Sikkim Himalayan Regional Centre (BSI, Gangtok).

Specimen vide No AB/01 has been deposited in herbarium of Botanical Survey of India (BSI, Gangtok) Sikkim Himalayan Regional Centre.

Extraction of plant material

The roots of *Euphorbia pulcherrima* were cleaned with water and shade dried until they become breakable and pulverized using an electric blender. The pulverized roots were kept in sealed container until further use. For the extraction powdered roots of *Euphorbia pulcherrima* (20gm) of powder was successively extracted with different solvents: petroleum ether, n-Hexane, ethanol, methanol and distilled water by the maceration method and filtered. Each filtrate was collected, concentrated, and dried under vacuum at 45°C using a rotary evaporator and kept in a desiccator at room temperature until further use.

Pharmacognostical Screening

The fresh roots were taken for macroscopical studies. Dried root powder was used for the study of microscopical and physicochemical studies, the roots of *Euphorbia pulcherrima* were cleaned with the water to remove the dirt and other foreign materials attached to the roots. They were shaded dried for two weeks until they become brittle and pulverized, using an electric blender. The powdered samples were stored in tight container until further use.

Organoleptic evaluation

Organoleptic evaluation refers to evaluation of the whole plant powder of the *Euphorbia pulcherrima*, by colour, odour, taste, texture, touch etc. The organoleptic characters of the sample were evaluated based on the method described by Siddiqui et al., 1995 [5].

Macroscopical evaluation

Macroscopic evaluation of *Euphorbia pulcherrima* root have been accomplished according to WHO Quality Control methods of herbal medicine. The colour, size, odor, shape, taste, surface and fracture of roots were observed[6]. Through magnifying lens internal and external structure of root was thoroughly observed. The colour and shape were verified by the visual examinations. The small section of plant root was squeezed between fingers and palms of the hands, using moderate pressure. First, the odour strength was determined (strong, distinct, none or weak) and then odour sensation (aromatic, rancid, fruity, mouldy, musty) was studied. Taste was distinctively determined (sweet, sour, pungent, mucilaginous, aromatic, astringent or bitter).

Powder microscopy

Powder microscopy was done following the standard procedure by collecting the crude drug and then washing thoroughly with water to remove unwanted matters, this is further air dried in the shade. After complete drying, it was powdered manually with the help of motor and pestle and then with electric blender. This was further subjected with different staining regents like, Sudan red, fast green, safranin, ruthenium, iodine solution etc.[7,8].

Physicochemical Investigation

The physio- chemical parameters such as loss on drying, ash values, pH value in 1% and 10% solution, aqueous, and alcoholic extractive values were carried out according to the methods recommended by the World Health Organization[9].

Loss on drying/ Moisture content

2gm of the powdered root of *Euphorbia pulcherrima* was accurately weighed in a tarred dish. For estimation of loss on drying, it was dried at 105°C for 5 hours in a hot air oven, cooled in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg per g of air-dried material[10].

Determination of alcohol-soluble extractive value and water-soluble extractive value

Alcohol-Soluble Extractive value:

About 5gm. of coarsely powdered, air-dried root plant was macerated with 1000 ml of solvent in a closed flask for 72 hours shaking frequently. Thereafter, it was filtered rapidly. Then filtrate was evaporated to dryness using rotary evaporator and then the remains was dried at 105°C and weighed. The percentage w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug.

Water Soluble Extractive value:

About 5 gm of the air-dried root powder is macerated with 100 ml of chloroform water in a close flask for 24 hrs. Shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Evaporate 25 ml of the filtrate to dryness in a tarred bottom flat bottom shallow dish dried at 105 ° C and weighed. The percentage of water-soluble extractive value was calculated with the reference to the air-dried drug [I.P. 1996].

Determination of total ash

The ash value was determined by taking accurately about 2 g of the powdered sample in a dried pre-weighed silica crucible and igniting until 6 hours in a muffle furnace at a temperature not exceeding 450° C until it is white, indicating the absence of carbon. Cooled in a desiccator and weighed. Total ash content was calculated in mg per g of air-dried material[11].

Determination of acid-insoluble ash

Twenty- five (25) ml of 1 N hydrochloric acid was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper (Whatmann 41) and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the heat to 550°C for 3 hours in a muffle furnace to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. Acid-insoluble ash content was calculated as mg per g of air-dried material.

Determination of water-soluble ash

Twenty- five (25) ml of water was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. Insoluble matter was collected on an ash less filter-paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C in a muffle furnace. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The weight of the residue was subtracted in mg from the weight of total ash. Water - soluble ash content was calculated as mg per g of air-dried material.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the petroleum ether, n-Hexane, ethanol and methanol extracts of root powder of *Euphorbia pulcherrima* were carried out using standard laboratory procedures, to detect the presence of different secondary metabolites (phytochemical constituents) such as

alkaloids, flavonoids, saponins, tannins, steroid glycosides, phenols, reducing sugars, protein and cellulose [11,12].

Determination of Phenolic compounds

Two to three drops of 1% ferric chloride (FeCl3) solution were added in to 2 ml portions (1%) of each extract. Phenolic compounds produce a deep violet colour with ferric ions.

Determination of Tannins

Ferric chloride test- A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride was added to the filtrate, formation of a blue-black, or green blackish colour in the presence of ferric chloride precipitate was taken as evidence for the presence of tannins.

Determination of Flavonoids

Alkaline reagent test- The crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Determination of Glycosides

Keller-kiliani test- Extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H2SO4. A brown ring at the interphase indicated the presence of cardiac glycoside.

Legal's test -(Test for cardenolides) To a clean test tube containing 1-2ml extract, add 1ml pyridine and 1ml sodium nitroprusside. Pink to red colour formation indicates the presence of cardenolides.

Determination of Alkaloids

Mayer's Test- One ml portions of each extract was acidified with 2-3 drops of 1M Hydrochloric acid and treated with 4-5 drops of Mayer's regent (Potassium Mercuric Iodide) Formation of a yellow or white coloured precipitate or turbidity indicates the presence of alkaloids.

Dragendroff's Test- Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's test- To a clean test tube containing 2-3 mL of extract, a few drops of Hager's reagent were added. The formation of reddish-brown precipitate indicates the presence of alkaloids.

Detection of Proteins

Biuret test- To a clean test tube containing 2ml of extract, add 4% NaOH and few drops of 1% CuSO4 solution. Formation of violet or pink colour indicates the presence of proteins.

Ninhydrin test- Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Detection of Saponins

Foam Test- 0.5 g of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Froth test-1gm sample was added to the test tube containing 5ml water and heated. Froth appearance indicates the positive result for saponins.

Detection of reducing sugar

Fehling's test- To a test tube 1 ml each a Fehling's A and B solutions were added and mixed. To this ~2 ml of plant extract was added and heated on a boiling water bath for ~10 minutes. Formation of brick red or orange precipitate indicates the presence of reducing sugar/ carbohydrates.

Pharmacological activity

Anti-bacterial assay

Cup Plate method was used for preliminary anti-bacterial activity against two bacterial strains (*Staphylococcus aureus* and *Escherichia coli)*. Gram positive and gram-negative bacteria respectively. A total of 100 µL of suspension of each microorganism containing approximately 0.2 mL was spread over each nutrient agar media for bacteria. Cups or cavities are made by using a sterile borer. After that the extract in different concentration is poured into the cups of agar plate and then incubated at 37 °C for 24hr. The zone of inhibition was measured as diameter in mm after 24-hr incubation at 37 °C [13,14].

RESULTS

Organoleptic properties of root of *Euphorbia pulcherrima*

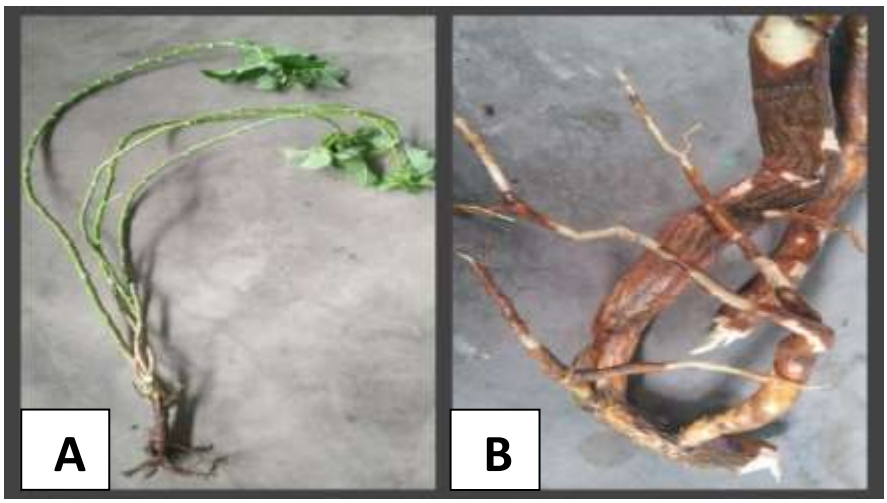

Figure 1: *Euphorbia pulcherrima*: **(a)** *Euphorbia pulcherrima* arial part **(b)** *Root of Euphorbia pulcherrima*

Table 1: Organoleptic evaluation of root of *Euphorbia pulcherrima*

Sl.no	Organoleptic characters	*Euphorbia pulcherrima*
01	Color	White
02	Odor	Pine-like terpenic scent, frequently with a mushroom note
03	Taste	Acrid, bitter, Starchy
04	Shape	Tuberous
05	Size	-
06	Fracture	Fibrous

Powder microscopy of pulverized root of *Euphorbia pulcherrima*

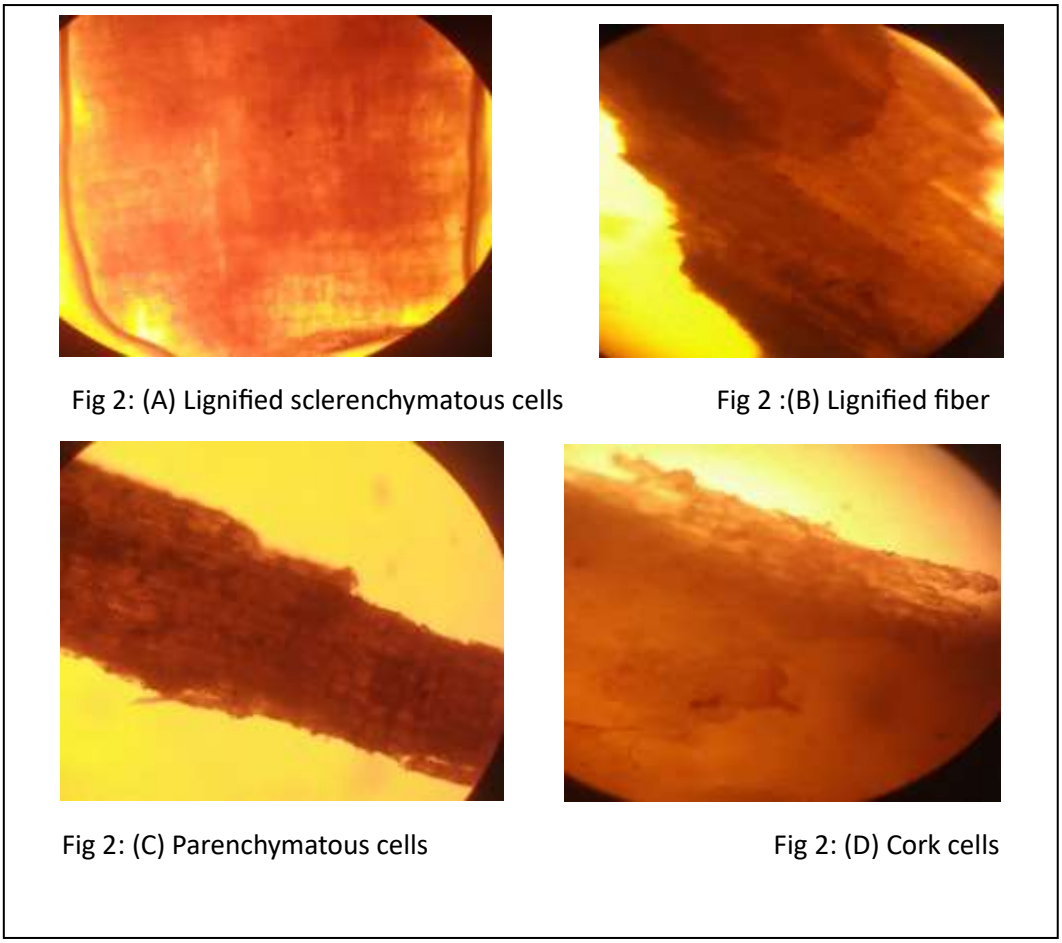

Figure 2: Powder characteristic of Euphorbia pulcherrima

Physio-chemical Analysis:

Table 2: Physiochemical Parameters of root of *Euphorbia pulcherrima*

Sl.no	Parameters	Values expressed in %W/W
01	Loss on drying	8.1%
02	Total ash	3%
03	Acid insoluble ash	2.2%
04	Water soluble ash	2.5%
05	Methanol soluble extractive value	0.13%
06	Water soluble extractive value	0.24%

Phytochemical screening:

Table 3: Phytochemical screening of ethanolic extract of root of *Euphorbia pulcherrima.*

Sl.no	Phytoconstituents	Tests	Observation
1	Alkaloids	Dragendroff's test	+
		Mayer's test	+
		Hager's test	+
2	Flavonoids	Alkaline reagent test	+
		Ferric chloride test	+
3	Carbohydrates	Fehling's test	_
		Barfoed's test	_
		Molisch's test	_
4	Glycoside	Keller-killiani test	_
		Legal's test	_
5	Cellulose	Iodine water test	_
6	Steroids	Salkwoski test	_
7	Protein	Biuret test	_
		Ninhydrin test	_
8	Tannins	Ferric Chloride test	+
		Vanillin Hydrochloride test	_
9	Saponins	Foam test	+
		Froth test	+
10	Phenols	Litmus paper test	_
		Ferric chloride test	_

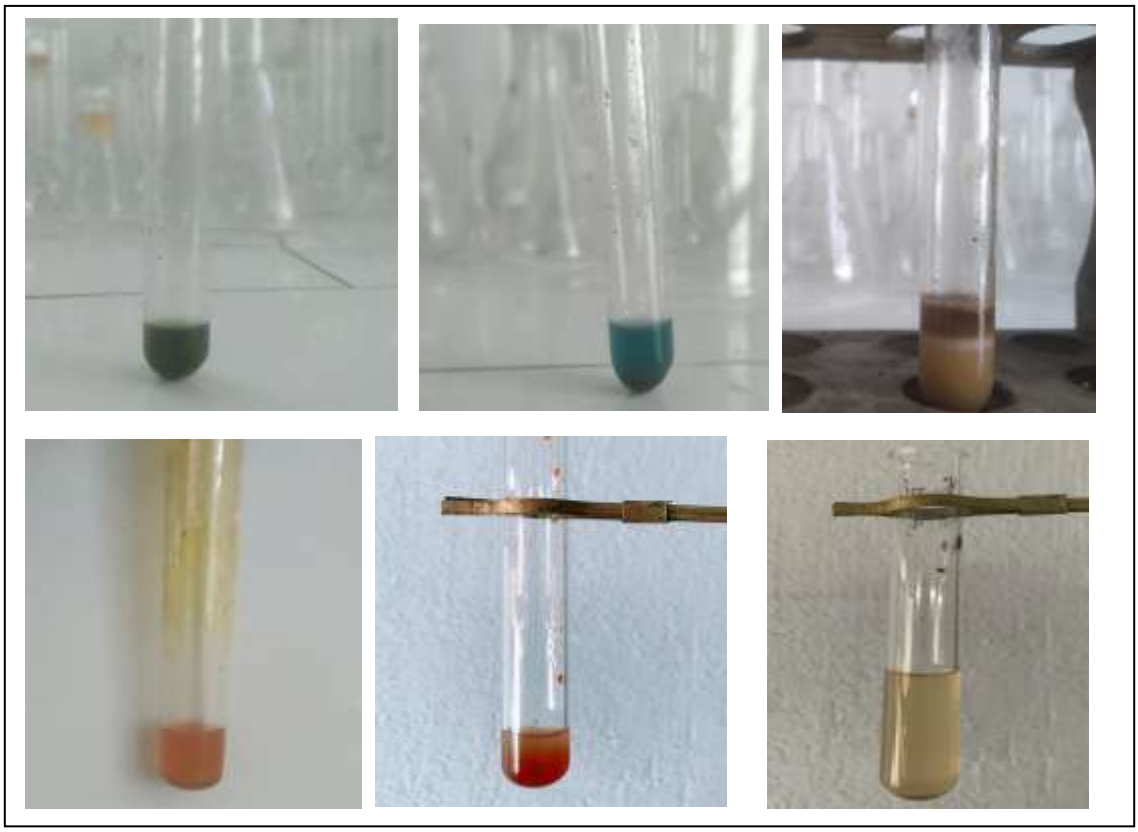

Figure 3: Phytochemical screening

Anti-bacterial assay

Table 4: Antibacterial activity of Ethanolic extract of *Euphorbia pulcherrima*

Sl.no	Extract	Dose mg/ml	Zone of inhibition Gm -ve bacteria (*E. coli*)	Zone of inhibition Gm +ve bacteria *(S. aureus)*
01.	Ethanol	2mg	5mm	12.5mm
		4mg	13mm	16mm
		6mg	14mm	17mm
		8mg	15mm	18mm

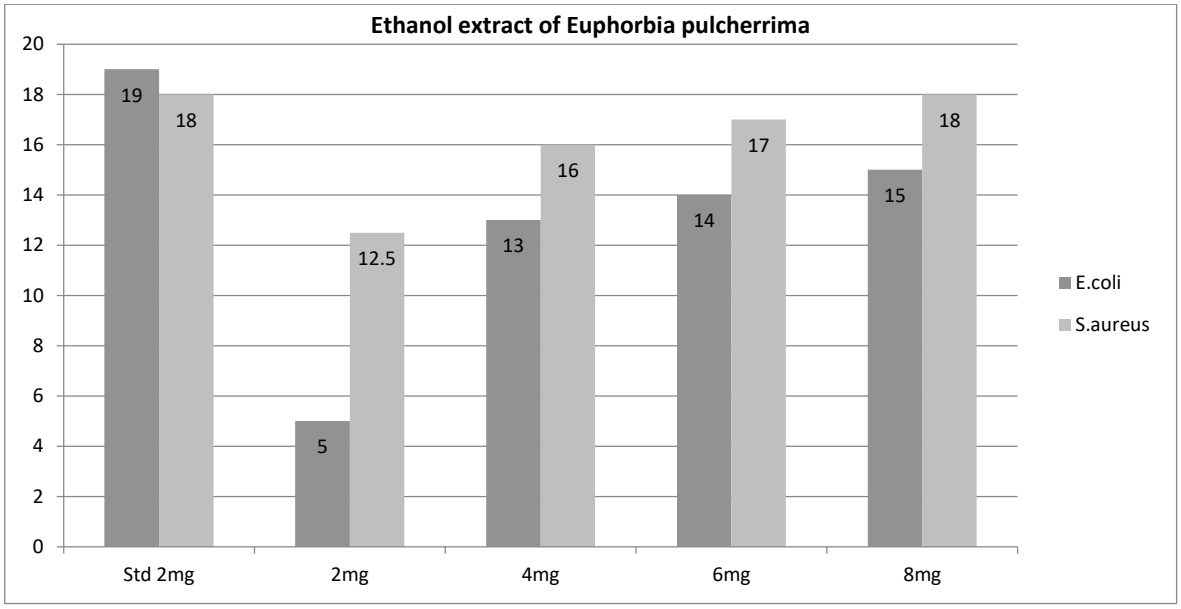

Figure 4: Graph representing the anti-bacterial activity of the ethanolic extract of *Euphorbia pulcherrima* on gram -ve and gram +ve bacteria.

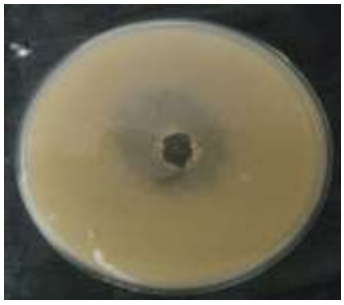

Fig 5: (A) Zone of inhibition of standard drug against Gram-negative bacteria *(E. coli*)

Fig 5 : (B) Zone of inhibition of Ethanolic extract against Gram-negative bacteria *(E. coli*)

Figure 5: Zone of inhibition against Gram-negative bacteria *(E. coli*)

Fig 6: (A) Zone of inhibition of standard drug against Gram-positive bacteria (*S. aureus)*

Fig 6: (B) Zone of inhibition of Ethanolic extract against Gram-positive bacteria *(*S. *aureus)*

Figure 6: Zone of inhibition against Gram-positive bacteria *(S. aureus)*

DISCUSSION

Standardization of medicinal plant, is required to ensure the quality of the drug, since substitutes or inferior substances maybe be used as herbal ingredient that aren't authentic. This analysis will aid in ensuring the identification, quality and the purity of the plant and its drug stability for human consumption. Several variables like microscopic, macroscopic analysis were conducted to investigate the study. These are the few techniques used which were cost-effective strategies for accurate identification of certain characters in plants.

Following this microscopic and macroscopic analysis whose results are detailed interpreted in table no 1 and fig no 2.

Other test which included powder microscopy using reagent like sudan red, safranine, ruthenium red, Fast Green, iodine brought us to the notice the presence of sclerenchyma, parenchyma, cork cells and lignified fiber, in the root extract of *E. pulcherrima.* Pharmacognostical evaluation like total ash value, water soluble ash and alcohol soluble ash was performed and the interpreted results are shown in table no 2 as follows with the percentage of 3 % (total ash value) which means on incineration *E. pulcherrima* was left with 3% of carbonate, phosphate and silicates of sodium, calcium, phosphates, and magnesium. Phyto-chemical analysis was performed as following the standard protocol using standard reagents which lead to the confirmation of phytoconstituents like alkaloids, flavonoids, and tannins better visual results are shown in fig no 3 and in table no 3. As phytochemical analysis revealed the presence of some specific constituents like Tannins, Flavonoids and alkaloids which lead to investigate further pharmacological test specifically anti- bacterial test as it is used in case of bruising, traumatic bleeding, and fractures according to the ethno-medical use of *Euphorbia Pulcherrima.* Anti- bacterial test was conducted using Cup plate method against gram negative bacteria (E. coli) and gram-positive bacteria (*Staphylococcus aureus)* results are tabulated in table no 4. The ethanolic plant extract showed highest activity against gram positive bacteria in dose dependent manner.

CONCLUSION

As there is not enough evidence for detailed physicochemical and phytochemical evaluation on root of *Euphorbia pulcherrima* is reported. Therefore, present work is taken up in the view to completely standardize the herb in accordance to parameters of World Health Organization (WHO) Guidelines and standard laboratory procedures. In the present study whole plant of *E. plucherrima* was thoroughly investigated for their organoleptic characters; physicochemical characters and major active constituents to analyse their quality, safety and standardization for their safe use. The present study reveals that the selected plant extract produces antimicrobial activity with dose dependent manner the observed activities of root extract might be attributed to the presence of secondary metabolites such as alkaloids, flavonoids and tannin compound. The further studies with purified constituents are needed to understand the complete mechanism of ani-bacterial activity. However, it needs further evaluation in clinical settings before consideration for the treatment of different diseases.

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Authors Contribution:

Anyash Basnett, Sushilta Pradhan, Rajat Das and Chandrika Sharma conceived and planned the experiments. Anyash Basnett and Rajat Das carried out the experiments. All the authors contributed to sample preparation. Anyash Basnett and Rajat Das contributed to the interpretation of the results. Anyash Basnett took the lead in writing the manuscript. All

authors provided critical feedback and helped shape the research, analysis and manuscript.

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Ethical Approval: NA

REFERENCES

1. Rauf A, Mohammad N. Phytochemical and pharmacological evaluation of aerial parts of Euphorbia pulcherrima L. WudpecherJornal of Pharmacy and Pharmacology. 2013; 2(2):15-20.)

2. Yakubu AI, Mukhtar MD. In vitro antimicrobial activity of some phytochemical fractions of Euphorbia pulcherimaL. (Poinsettia). Journal of medicinal plants research. 2011 Jun 18; 5(12):2470-5.)

3. Dai Y, Liu S, Xu J, Zhao C, Gu Q. Triterpenoids from Euphorbia pulcherrima with inhibitory effects on osteoclastogenesis. Fitoterapia. 2019 Apr 1; 134:355-61. https://doi.org/10.1016/j.fitote.2019.02.028

4. Kurian JC. Plants that heal. Pune, India: Oriental Watchman Publishing House; 1995. p. 134-56

5. Siddiqui & M.A. Hakim, Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, anuary, Central Council for Research in Unani Medicine (CCRUM), New Delhi, (appendix), 1995, 24-25

6. Shewale S, Undale V, Shelar M, Pimple B, Kuchekar M, Bhalchim V. Macroscopic and Microscopic Evaluation of Sansevieria cylindrica Plant. Pharmacognosy Research. 2022; 14(4). https://doi.org/10.5530/pres.14.4.60

7. Anbazhahi T., Kadavul K., Suguna G., Petrus A.J.A. Studies on the pharmacognostical and in vitro antioxidant potential of Cleome gynandra Linn. Leaves. Nat Prod Radiance. 2009; 8(2):151-7)

8. Gupta P, Vasudeva N, and Sharma S.K. Pharmacognostical Study and Preliminary Phytochemical Screening of the Roots of Tagetes erecta Linn. Roots. Hamdard Medicus 2009; 52(1).

9. World Health Organization. organization mondiale De La santé, Quality control methods for plant materials, 559. Rev.; 1:08-67.

10. Choudhary N, Sekhon BS, An overview of advances in the standardization of herbal drugs, J. Pharm Educ. Res., 2011; 2(2):55-70.

11. Kokate CK, Khandelwal KR, Pawer AP, Gokhale SB, Practical Pharmacognosy, 3rd edition, Nirali Prakashan, Pune, 1995, 137

12. Farnsworth NR, Biological and Phytochemical screening of Plants, Journal of pharmaceutical Science, 1996; 55:225-276 https://doi.org/10.1002/jps.2600550302

13. Tolulope O. Phytochemical Screening, Antibacterial Activity and Fatty Acids from Heliotropium Indicum. Pharmacognosy Journal. 2023; 15(2). https://doi.org/10.5530/pj.2023.15.53

14. Tofighi Z, Molazem M, Doostdar B, Taban P, Shahverdi AR, Samadi N, Yassa N. Antimicrobial activities of three medicinal plants and investigation of flavonoids of Tripleurospermum disciforme. Iranian journal of pharmaceutical research: IJPR. 2015; 14(1):225.