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EVALUATION OF IN-VITRO ANTIBACTERIAL, ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF EUPHORBIA GENICULATE LEAF EXTRACT

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Abstract

The aim of the present study was to investigate the phytochemical profile as well as the antioxidant, anti-bacterial and anti-inflammatory properties of Euphorbia geniculate leaf extracts. Most traditional natural products are getting more popular. Natural products continue to produce bioactive agents because of the amazing chemical diversity that is readily available. They were assessed as potential therapeutic candidates for the treatment of infectious illnesses in both humans and animals. The spurge family, Euphorbiaceous, is well-known among plant families due to scientific evidence supporting its antiviral, antibacterial, anticancer, cytotoxic, and antitumor characteristics.

Keywords: Euphoria geniculata, Ibuprofen, Fluconazole, Anti-inflammatory Activity, Anti-bacterial activity.

Introduction

Euphorbia (Euphorbiaceae) is the third most common genus among flowering plants. It is widespread around the world with more than 2000 species and with an exceptional diversity such as shrubs, vines, and grassy plants. *Euphorbia* is well known, since ancient times, for its therapeutic activity on several gastrointestinal ailments, infections, skin irritations, body pain, microbial illness, sensory disorders, and as an antidote against snake venom. Numerous species are commonly cultivated for ornamental purposes. Medicinal herbs have long been used to treat a variety of illnesses. The use of medicinal plants for illness treatment and management is a less expensive and more readily available alternative to pricey synthetic medications that are frequently in short supply. Ethnobotanical recording of medicinal plant use is a good way to find possible novel medication sources in most cases. Because important plant species for pharmacological studies are easily recognized, the use of ethnobotanical expertise in drug discovery is gaining popularity. New chemical compounds having medicinal potential have a better chance of being discovered. Natural reservoirs of pharmacologically active chemicals that can be used medicinally are medicinal plants. Many effective medications have been discovered as a result

of research on traditional medicinal plants.

As in other nations that are developing, medicinal plants continue to be one of the most crucial therapeutic tools in Egyptian traditional medicine. The identification of novel compounds with a variety of therapeutic qualities is greatly enhanced by the flora of Egypt. Numerous studies have documented the antibacterial properties of Egyptian medicinal plants. The Euphorbiaceae is a big family with around 300 genera and 7000 species that are found all over the world. The Euphorbiaceae is one of the largest dicotyledon families, ranking fourth after the Compositae, Leguminosae, and Rubiaceae. This family contains a large number of taxa and species. Euphorbia is a kind of plant. Medicine is made from the portions of the plant that grow above ground. Breathing diseases such as asthma, bronchitis, and chest congestion are treated with euphorbia. Mucus in the nose and throat, throat spasms, hay fever, and tumors are all treated with it. Many Euphorbia species have been utilized as anticancer medicines in the past. The word "anthelmintic" is from the Greek word, "anti" which suggests "against" and "helminths" means "worm" which implies "to kill or wipe out worms or parasites". Anthelmintics are drugs that either kill (vermicide) or) expel (vermifuge infesting helminths. The helminths are worm-like parasites. The clinically relevant groups are separated in line with their general external shape and therefore the host organ they inhabit. There are both hermaphroditic and bisexual species. The definitive classification relies on the external and internal morphology of egg, larval, and adult stages. Helminthic worms are highly prevalent and, betting on the species, may existas free-living organisms or as parasites of plant or animal hosts. (Peter F. Weller Introduction to Helminthic Infections) Healthcare workers in tropical and sub-tropical settings where strongly loidiasis is prevalent or caring for patients who have travelled to such areas, have to maintain a high level of awareness about the utilization of corticosteroids, including when this class of anti-inflammatories is given to patients suspected of infection with SARS-CoV-2.2. An antioxidant could be a molecule capable of inhibiting the oxidation of other molecules. Oxidation could be a chemical process that transfers electrons or hydrogen from a substance to an oxidizer. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing atom intermediates and inhibiting other oxidation reactions. They are doing this by being oxidized themselves, so antioxidants are often reducers like water-soluble vitamins or polyphenols. A diet high in antioxidants may reduce the risk of many diseases (including heartdisease and certain cancers). Antioxidants scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation. The protective effect ofantioxidants continues to be studied around the world. Antioxidants neutralize free radicals by giving up some of their own electrons. In making this sacrifice, they act asa natural off switch for the free radicals. This helps break a chain reaction that can affect other molecules in the cell and other cells in the body.

Inflammation usually occurs when infectious microorganisms such as bacteria, viruses, or fungi invade the body, reside in particular tissues, and or circulate in the blood. Inflammation may also happen in response to processes such as tissue injury,cell death, cancer, ischemia and degeneration. Mostly, both the innate immune response as well as the adaptive immune response is involved in the formation of inflammation. The innate immune system is the foremost defense mechanism against invading microorganisms and cancer cells, involving the activity of various cellsincluding macrophages, mast cells, and dendritic cells. The adaptive immune systems involve the activity of more specialized cells such as B and T cells who are responsible for eradicating invading pathogens and cancer cells by producing specific receptorsand antibodies. Numerous inflammatory mediators are synthesized and secreted during inflammatory responses of different types. Inflammatory substances are usually divided into two main categories: pro- and anti-inflammatory mediators. Nevertheless, some mediators such as interleukin (IL)-12 possess both pro-and anti-inflammatory properties. Among the inflammatory mediators and cellular pathways that have been extensively studied in association with human pathological conditions are cytokines (e.g., interferons, interleukins, and tumor necrosis factor α), chemokines (e.g., monocyte chemoattractant protein 1), eicosanoids (e.g., prostaglandins and leukotrienes) and the potent inflammation-modulating transcription factor nuclear factor. Antibacterial, as well as antiviral activity of a molecule, is completely associated with the compounds that provincially kill bacteria and viruses or slow down their rate of growth, without being extensively toxic to nearby tissues. Most recently discovered antimicrobial agents are modified natural compounds and this modification is done through chemical mode, for example, b-lactams (penicillin's), carbapenems, or cephalosporin. Pure natural products, such as aminoglycosides, and other entirelysynthetic antibiotics, for example, sulfonamides, are also frequently used. The antimicrobial agents could be classified as agents that can either be bactericidal, whichkills bacteria, or bacteriostatic, which slows down the growth of bacteria. Antibacterialagents are the most important in fighting infectious diseases. But, with their wide use as well as abuse, the appearance of bacterial resistance toward antibacterial agents has become a major problem for today's pharmaceutical industry. Resistance is most commonly based on developmental processes taking place, for example, antibiotic therapy, that leads to inheritable resistance. This increasing resistance of the microorganisms toward antibacterial agents has been responsible in recent years for serious health issues. Most infectious bacteria are resistant to a minimum of one of the antibiotics that are generally used to eliminate theinfection. This problem motivates the study of new agents that can efficiently inhibit the growth of microorganisms. The literature survey revealed that Euphorbia geniculata is native to all Indian regions. It is a medicinal herb with extreme ethno medicinal properties used to treat coagulation, viral infection, and laxative, diabetic, wound healing. The herb contains alkaloids, flavonoids, tannins, saponins, steroids are the major bioactive components. So, keeping all these things in view, the present study was planned to evaluate the antioxidants, antiemetic's, anti-inflammatory and antibacterial potential of Euphorbia geniculata. The objective, literature review, methodology, results discussion, conclusions, summary and bibliography are recorded in the following sections of this project.

Material and Methods

List of Chemicals Manufacturer

Euphoria geniculata extract, Ibuprofen, Fluconazole, Methanol, Ethanol, Diclofenac sodium, Albendazole.

List of Instruments

Laminar air flow, Bacteriological incubator, Spectro photometer, UV visible spectrometry

Preliminary phytochemical screening

Antioxidant Activity

DPPH radical scavenging assay.(2,2 diphenyl -1-picrylhydrazyl)

Anti-inflammatory

Antibacterial Activity:

Preliminary phytochemical screening-

I. Preliminary Test:

Colour- greenish black

Odor- Pungent

Taste- bitter

A. Detection of Carbohydrates (Benedict test):

Approximately 1 ml of sample is placed into a clean test tube.2 ml (10 drops) of Benedict's reagent (CuSO4) is placed in the test tube. The solution is then heated in a boiling water bath for 3-5 minutes.

Observe for color change in the solution of test tubes or precipitate formation.

B. Detection of protein (Millions test):

In test-tube add 2 ml of protein into separate labeled test tubes. Add 3-4 drops of Millon's reagent, and immerse the tubes in a boiling water bath for 5 minutes.Cool the tubes and record the colors formed.

C. Detection of Alkolides:

Take a clean dry test tube. Take 2 ml Deagendroff reagent .add 2 ml extract.

D. Detection of flavonoids:

Take a clean dry test tube .Take 2 ml extract and add 2 drop of sulphuric acid.

E. Detection of Tannins:

Take test tube with ferric chloride solution .Add 2 ml extract.

F. Detection of saponins:

Shake aq solution of sapponing containing sample producing foam which isstable for 15 min or more.

G. Detection of steroids:

Take clean and dry test tube mix with 2ml test extract with acetic anhydride. Boil and cool .Add 0.5 ml H2so4

II. Pharmacological screening of plant extract:

Pharmacological Screening of plant extracts: In vitro study of Euphoria and leafextracts was done which incorporates.

1. Anti-oxidant activity
2. Anthelmintic activity

3. Anti-inflammatory Activity

4. Antibacterial activity

Fig. No. 1 Euphoria geniculata Leave

1. In-vitro Anti-oxidant activity:

An antioxidant could be a molecule capable of inhibiting the oxidation of other molecules. Oxidation could be a chemical change that transfers electrons or hydrogen from a substance to a chemical agent, Oxidation reactions can produce free radicals. Inturn, these radicals can start chain reactions. When the chain reaction occurs in an exceedingly cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing radical intermediates, and inhibit other oxidation reactions.[9]

2) Diphenyl-1picryl-hydrazylradical scavenging (DPPH) Activity Principle:

DPPH may be a stable radical and is widely wont to assess the novel scavenging activity of antioxidant compounds. This method relies on the reduction of DPPH in methanol solution within the presence of a hydrogen donating antioxidant because of the formation of the non-radical form DPPH-H. This transformation leads to a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The atom scavengingactivity is measured by using 1, 1- diphenyl-2 picryl-hydroxyl Vani T.Rajani,Met(1997).

Reagent Required-

1) DPPH
2) Pure Methanol

Preparation of samples and standard solutions:

Accurately weighed 10 mg of Methanolic and Aqueous extracts and therefore standard antioxidant and dissolved separately in 10 ml of phosphate buffered saline. These solutions were serially diluted with methanol to Procedure: get the lower dilutions".

Evaluation of Antioxidants Activity:

Antioxidant activity of ethanol extract of *Euphorbia geniculata* were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl- Hydroxyl) free radicals (George et al., 1996).

100µL of ethanol extract of *Euphorbia geniculata* taken in the microtiter plate. 100µL of 0.1% Methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490nm [2] Radical scavenging activity was calculated by the following equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100.

2. In-vitro Anthelmintic activity:

Animal used:

Indian adult earthworms (Pheretima Posthuma) were accustomed study anthelmintic activity. The earthworms were collected from moist soil and washed with normalsaline to get rid of all excrement. The earthworms of 3-5 cm long and 0.1-0.2 cm in breadth were used for all experimental protocol. The earthworm resembles

both anatomically and physiologically to the intestinal roundworm parasites of men, hence may be wont to study the anthelmintic activity.

Evaluation of anthelminthic Activity:

Principle- Anthelmintic are drugs used for treating parasitic infections. They kill parasites by: Binding to nerves and muscle cells and causing paralysis and eventually death of the parasite. Blocking the transport of glucose by the cells and thus causing paralysis of the parasite.

Indian adult earthworms (pheretima Posthuma) were used to study anthelmintic activity. The earthworms (collected from the water-logged areas of soils, Sangli,Maharashtra) were washed with normal saline to remove all fecal materials. Theearthworms in 4-5 cm. in length and 0.1 - 0.2 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study anthelmintic activity. The ethanolic extracts of *Euphorbia geniculata* were tested for anthelmintic activity. Pheretima Posthuma of nearly equal size were selected randomly for present study.The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into four groups of six earthworms in each. Albendazole diluted with normal saline solution to obtain 10mg/ml served as standard and poured into Petri dishes. The extracts were dissolved in small quantity of 10mg/ml DMSO and adjusted the volume up to 15 ml with normal saline solution. The extract was evaluated by the time taken for complete paralysis and death of earthworms. The meanlethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive. The mean lethal time for paralysis and death of the earthworms for different test extracts and standard drug are tabulated.

3. In-vitro Anti-inflammatory Activity:

Principal:

Nonsteroidal anti-inflammatory drugs (NSAIDs) are medications that relieve or reducepain. The most popular examples of drugs in this group are aspirin and ibuprofen. NSAIDs come under the wider definition of non-opioid analgesics.

4. In-vitro Antibacterial Activity:

Antibacterial study The antibacterial activity of the plant extracts was tested against four Gram-positive bacteria, Bacillus stereothermophillus, B. subtilis , Salmonella Typhi , Staphylococcus aureus and Gram-negative bacteria.[10]

Evaluation of Antimicrobial Activity-

The inoculum of the microorganism was prepared from the bacterial cultures. 15ml of nutrient agar(Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify. 100µl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly. Wells of 6mm in diameter were bored using a sterile cork borer. Solutions of all the compounds(5Mg / ml and 10 mg /

ml) in DMSO were prepared.100 µl of plant extracts solutions was added to the wells. The petri plates incubated at 370C for 24 h. streptomycin (1mg/ml) was prepared as a positive control DMSO was taken as negative control. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI) all the determination were performed in triplicates.

RESULT

1. Phytochemical screening of Euphorbia geniculate:

Table No. 01: Preliminary Phytochemical screening of *Euphorbia geniculata* leafextract.

Sr. no.	Test	Present (+)	Absent (-)
1	**Test for carbohydrate**	+++	-
2	**Test for protein**	++	-
3	**Test for alkaloids**	+	-
4	**Test for flavonoids**	++	-
5	**Test for tannins**	+++	-
6	**Test for saponins**	++	-
7	**Test for steroids**	++	-

Note: +++ High intensity, ++ Medium intensity, + Low intensity.

The above observation table shows the presence of phytoconstituents in the extracts. The extract contains carbohydrates, steroids, tannins, flavonoids, saponins, proteins, and alkaloids.

1. In-vitro Antioxidant activity by DPPH (96 well method):

Table No.02: Effects Of Compounds Against DPPH Free Radical By 96 Well PlateMethod

Sr. no	Compounds	ABS or OD at 490nm	DPPH radical scavenging activity(%)
1.	Control	**3.486**	
2.	Std Ascorbic acid (1000µg/ml)	1.430	58.97
3.	Ethanolic extract of *Euphorbia geniculata* (500 µg/ml)	2.250	35.45
4.	Ethanolic extract of *Euphorbia geniculata* 10mg/kg (1000 µg/ml	2.061	40.87

5	Ethanolic extract of *Euphorbia geniculata* (1500 µg/ml)	2.340	44.12

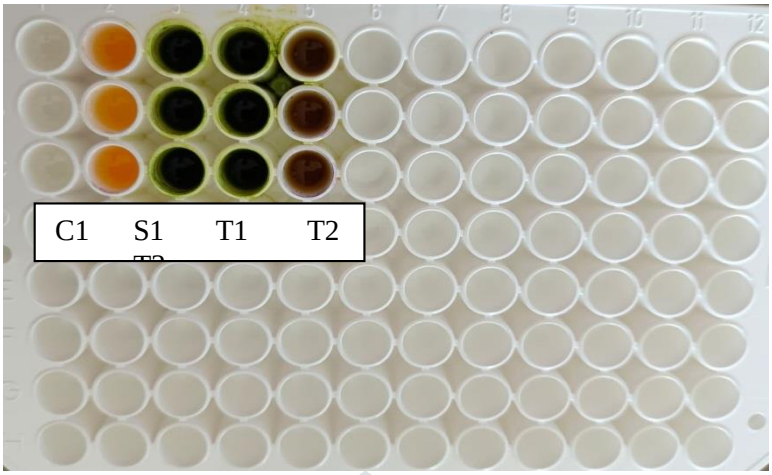


Fig.no.2 .Effects of Compounds against DPPH Free Radical by 96 WellPlate Method

Anthelmintic activity:

Table No. 03: Anthelmintic Activity of Ethanol Extract of *Euphorbia geniculata*

Sr no.	Compound details	Concentration mg/ml	Average Time in (min)	
			Paralysis Time(min)	Death Time (min)
1	Blank (Distilled water)	----	----	----
2	Standard (Albendazole)	10	2.00	3.00
3	Ethanol extract of *Euphorbia geniculata*	20	5.00	9.00
4	Ethanol extract of *Euphorbia geniculata*	40	**5.27**	**7.00**

Fig no. 03: Euphorbia geniculata leaf extract with Pheretima Posthuma

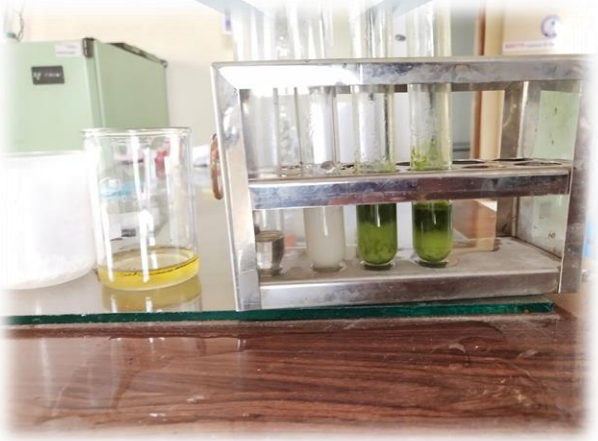
Fig No. 04: Albendazole Solution with Pheretima Posthuma

1. In-vitro Anti-inflammatory activity:

Table no .4: In vitro anti-inflammatory activity by Protein denaturation method.

Sample code	Concentration	Anti-inflammatory activity	
		Absorbance at 660nm	% inhibition
Blank		**1.83**	
Diclofenac sodium	**1 mg/ml**	0.89	51.36
Ethanol extract of *Euphorbia geniculata*	**5mg/ml**	1.13	38.35
Ethanol extract of uphorbia geniculate	**10mg/ml**	1.08	40.98

Fig no. 05: Inhibition of protein denaturation method



2 In-vitro Antibacterial Activity:

Table No.5: In-Vitro Antibacterial Activity of Extract against Salmonella Typhi

Sr.No	SAMPLES	CONCENTRATION (mg/ml)	ZONEINDIAMETER (mm)
1	Control	-	**0**
2	Standard (Streptomycin)	1	**20**
3	Ethanol extract of (5mg/ml)	5	**16**
4	Ethanol extract of (10mg/ml)	10	**18**

At the concentration 5mg/ml and10mg/ml, the SAMPLE showed good activityagainst.Salmonella Typhi:

Fig no. 6: zone of inhibition of Salmonella Typhi **Antibacterial Activity**

Escherichia coli:

Table No. 6: In-Vitro Antibacterial Activity of the Extract against Escherichia coli

Sr. No.	SAMPLES	CONCENTRATION (mg/ml)	ZONE IN DIAMETER (mm)
1	Control	-	0
2	Standard (Streptomycin)	1	19
3	Ethanol extract of (5mg/ml)	5	09
4	Ethanol extract of (10mg/ml)	10	**11**

At the concentration 5mg/ml and10mg/ml, the SAMPLE showed good activity against

Escherichia coli:

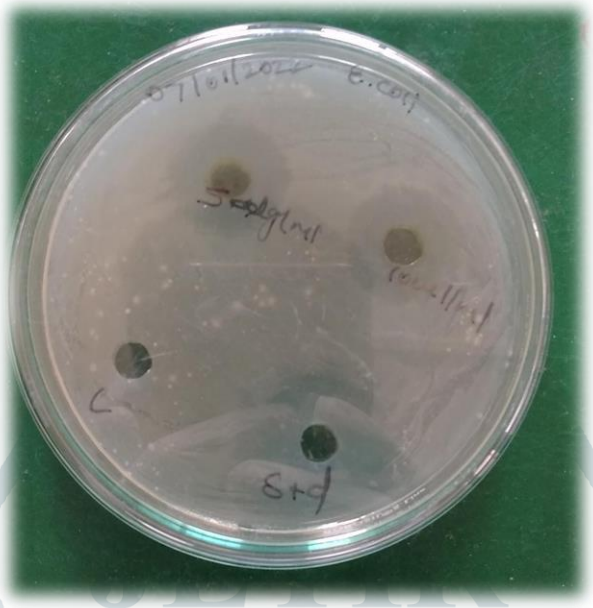


Fig no. 07: zone of inhibition of E. coli

Antibacterial Activity Bacillus subtilis:

Table No. 7: In-Vitro Antibacterial Activity Of The Extract Against *BacillusSubtilis.*

Sr. No.	**SAMPLES**	**CONCENTRATION (mg/ml)**	**ZONE IN DIAMETER (mm)**
1	Control	**-**	**0**
2	Standard (Streptomycin)	1	**29**
3	Ethanol extract of (5mg/ml)	5	**16**
4	Ethanol extract of (10mg/ml**)**	**10**	**17**

At the concentration 5mg/ml and10mg/ml, the SAMPLE showed good activityagainst.

Bacillus subtilis:

Fig no. 08: zone of inhibition of Bacillus subtilis

Antibacterial Activity Staphylococcus Aureus:

Table No. 8: In-Vitro Antibacterial Activity Of Extract Against Staphylococcus Aureus.

Sr. No	SAMPLES	CONCENTRAON (mg/ml)	ZONEINDIAMETER (mm)
1	Control	-	**0**
2	Standard(Streptomycin)	1	**22**
3	Ethanol extract of (5mg/ml)	5	**14**
4	Ethanol extract of (10mg/ml)	10	**16**

At the concentration 5mg/ml and10mg/ml, the SAMPLE showed good activityagainst.

Staphylococcus Aureus:

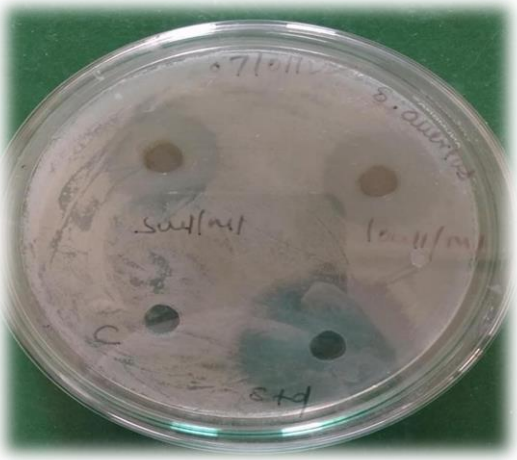


Fig no. 09: zone of inhibition of staphylococcus aureus

Result and Discussion:

Today, the medicinal plants have become more important in primary health care, because of their secondary metabolites which may play copious biological activities, against cancer and infectious disease· About 70% of population of all the world use traditional medicines derived from plant species for their treatment. Euphoria geniculata commonly known as Euphoria. It is widely recognized in ayurvedic systemof Indian medicine as tonic previous studies reported anti-inflammatory, antimicrobial, antioxidant, one of all the predominant effects of anthelmintic drugs like piperazine, praziquantel on the Helminthes is to cause a neurological disease by affecting neurosynapses and motion of worms that lead to expulsion of paralysis by peristalsis. Albendazole acts by blocking glucose uptake and depletion of its glycogen stores thus decreasing ATP formation; it binds to free protein within the digestive tube of the host animal or glycoprotein on the cuticle of parasite resulting in gradual loss of intracellular microtubules within the cell of the worm through inhibition of tubulin.

The in vitro Antibacterial activity of Euphoria geniculata against staphylococcus aureus, Salmonella Typhi, Bacillus subtilis and E.coli was passed by well diffusion method by measuring the inhibition zone. According the result presented in tables Euphoria geniculata leaf extract produced inhibition zones larger than mm these results demonstrated that the Euphoria geniculata leaf extract has potent antibacterial activity against staphylococcus aureus, Salmonella Typhi, Bacillus subtilis and E.coli.

The preliminary phytochemicals screening shows the presence of phytoconstituents in the extracts. The extract contains carbohydrates, steroids, tannins, flavonoids, saponins, proteins, and alkaloids. This phytoconstituents are present in the Euphoria geniculata leaf extract. According to effect of Antioxidant Activity table content Ethanol extract of *Euphorbia geniculata* (500 µg/ml & 1000 µg/ml) show good inhibition of free radical scavenging like DPPH as compared to standard drug Ascorbic Acid

The effect of anthelmintic activity exhibited by extracts on Pheretima Posthuma is shown in Table Closer inspection of data from this table indicates that ethanolic extracts 20mg/mL,40mg/mL showed very high activity as compared to standard drug of Albendazole solution. At Antibacterial activity concentration of 5mg/ml and10mg/ml the sample of Euphoria geniculata showed good activity against Salmonella Typhi, E. coli. Bacillus subtilis, staphylococcus aureus as compared streptomycin. According to the effect Auto inflammatory Activity table It indicates that the 10mg ethanol extracts showed good inhibition of protein denaturation as

compared to standard drug of Diclofenac sodium.

Conclusion

Euphoria geniculata from Euphorbiaceae family and the present study is carried out to evaluate the Anthelmintic activity, Antioxidants activity, Antibacterial activity and Anti-inflammatory activity. The Screened Euphoria geniculata leaf extract contained Alkaloids, Tannins, flavonoids, steroids, saponins proteins in high concentration, the matter of anthelminticresistance, toxicity, and therefore the increasing concern over the presence of drugs in animal products has led to a renewal of interest within the use of plant-based drugs, plant materials evaluated within the current study had been identified from various sources to function anthelmintic agents by traditional healers, Our current in vitrostudy examines the anthelmintic activity of *Euphorbia geniculata* leaf extract in pheritima Posthuma earthworms was chosen to fulfill the way utilized by people in traditional medicine. Thecurrent study showed 100% efficacy of the plant extract against the Helminthes at the concentration of 100mg/ml. which is that the highest efficacy value and was comparable the quality anthelmintic. The info obtained from anthelmintic effect of Euphoria geniculata extract showed that 100 mg of ethanolic extract show significant anthelmintic activity which is most significant ($P<0.01$) When compare to manage. Ethanolic extract of Euphoria geniculata extract demonstrated death of worms in less time as compared to Albendazole especially At standard concentration of 10mg/ml. Aqueous extract show dose dependent paralysis starting from loss of motility to loss ofresponse to external stimuli in a very significant manner $P<0.01$). The combined doseof 100mg/ml show highly significant result and ($P<0.001$).

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