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Phytochemical and Pharmacological Evaluation of *Euphorbia hirta Linn*.Leaves for Aphrodisiac Activity.

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ABSTRACT
Sexual dysfunction is very serious problem in human being. Inability to achieve normal sexual intercourse called sexual dysfunction, which include premature ejaculation, retrograded, retarded or inhibited ejaculation, erectile dysfunction, arousal difficulties (reduced libido), compulsive sexual behavior, orgasmic disorder, failure of detumesence in males and Desire disorders, Arousal disorders, Orgasmic disorders, Sexual pain disorders in females. The trends of herbal medicine is running in the market to cure specific diseases due to its less adverse drug reaction, toxicity and other injurious effects. There is huge variety of plant which capable to cure and treat specific type of disease. Researchers further studying to identify plant activity. Many plants have ability to correct sexual dysfunction in male and female. Among them some plants Panax quinquefolius L. (American giseng), Eurycoma longifolia, Corynanthe yohimbe, Maca, Ginkgo biloba, Turnera diffusa (Damiana), Terminalia catappa L., Tribulus terrestris, Euphorbia Hirta L., Passiflora incarnata L., Ptychopetalum olacoides, Cnidium monnier are commonly used Herbs to correct sexual dysfunction in male and female. In this articale we are studies about the Euphorbia Hirta L.

Key Words; *Euphorbia hirta L, Erectile dysfunction, Aphrodisiac activity*

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I. Objectives:

Erectile dysfunction is major health problem, the consequences of which can affect both the man and his family. There has been worldwide increase in the incidence of ED, probably due to aging population and other risk factors such as the presence of chronic illnesses (e.g. heart diseases, hypertension and diabetes mellitus), smoking, stress, alcohol, drug abuse and sedentary lifestyle. Many synthetic drugs are available to treat ED but, they are expensive and can provoke fatal adverse effects. Since, many people are now relying on herbal medicines for health care, so there should be renewed interest towards the search for traditional herbs, which are being constantly claimed for treatment of sexual dysfunction. Literature survey revealed that use of *Euphorbia hirta Linn.* by tribes in Satpuda region, In treatment of cough, asthma, diarrhea, piles and semen debility, worm infection, leprosy, skin diseases, urinary infection and disurea.It is aphrodisiac and enriches blood etc. Since no scientific evidence on aphrodisiac activity is reported, way of investigation would be carried out to evaluate its aphrodisiac potential. Thus, the objective of this study is to evaluate phytochemical and pharmacological activity of *E. hirta Linn*. for aphrodisiac activity.

II. Introduction of Erectile Dysfunction.[1,2]

Human sexuality, general term referring to various sexually related aspects of human life including physical and psychological development, behavior, attitude and social customs associated with the individual's sense of gender, relationships, sexual activity, mate selection and reproduction. Sexuality permeates many areas of human life and culture thereby setting human apart from other members of animal kingdom, in which the objective of sexuality is more often confined to reproduction.[1] All living things reproduce. Reproduction- the

process by which organism makes more organisms like themselves- is one of the things that set living things apart from nonliving things. But even though the reproductive system is essential to keep a species alive, unlike other body system it is not essential to keep an individual alive.

One of the main aim of marriage is the procreation (reproduction) and more importantly for sexual fulfillment of both partners. For life to continue, an organism must reproduce itself before it dies. In *Homo sapiens*, reproduction is initiated by the mating of a male with a female in sexual intercourse which facilitates the coming together of sperm and egg for the purpose of fertilization. For there to be a normal sexual intercourse and sexual fulfillment in males, the male sexual organ (the copulatory organ, the penis) and factors relating is a major problem facing the reproduction process. This is known as sexual dysfunction. This condition which is various types can be managed by the use of aphrodisiacs.

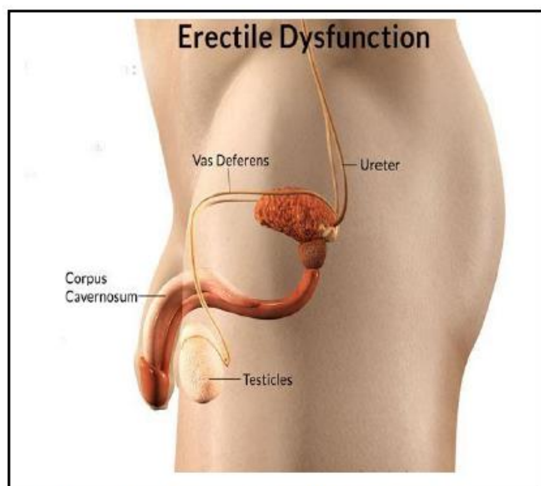


Fig-1.Anatomy of Erectile Dysfunction

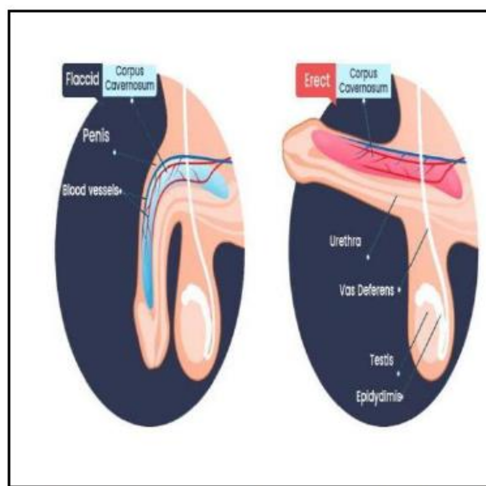


Fig-2. Anatomy of Erection & Flaccid

2.1. Male Sexual Function[2]

Sexuality is a complex, multi-dimensional phenomenon that incorporates biological, psychological, interpersonal and behavioral dimension. Sexual behavior in male rats consists of three different phases:

- **Mount:** The animal assumes the copulatory position, but does not insert its copulatory organ (the penis) into vagina.
- **Intromission:** The copulatory organ enters the vagina during mounting.
- **Ejaculation:** Forceful expulsion of semen.

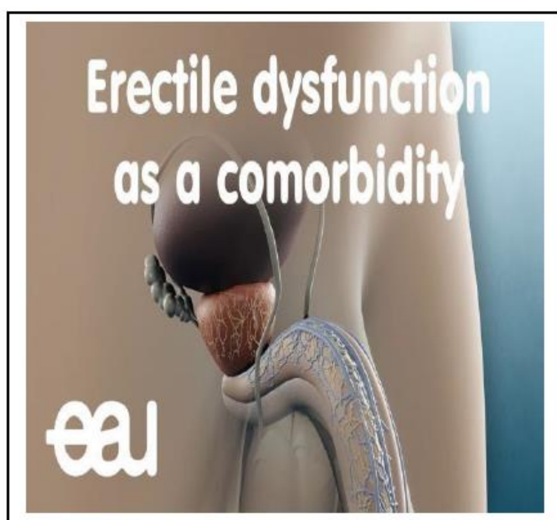


Fig-3. Erectile Dysfunction as acomorbidity

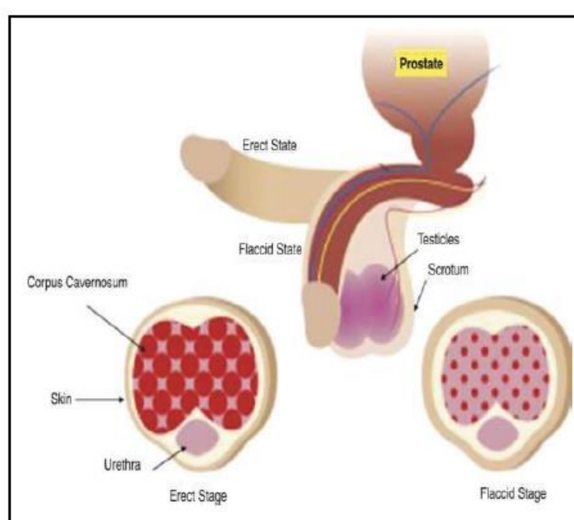


Fig-4.Erectile stage and Flaccid Stage inmale

- Sexual stimulation of the human male results in a series of psychological, neuronal, vascular, and local genital changes. At least three different classifications for these changes have been described. Some authors described a psychosexual response cycle that consist of four phase: excitement, plateau, orgasm and resolution. Another classification is based on penodynamic changes during the sexual cycle divided each of the psychosexual phases into two interrelated events as excitement into latency and tumescence: plateau into erection and rigidity; orgasm into emission and ejaculation; and resolution into detumescence and refractoriness. A third classification focuses on the functional activities during the sexual cycle by adding an initial phase of desire or libido. Thus, the normal male sexual response cycle can be functionally divided into five interrelated events that occur in a define sequence: libido, erection, ejaculation, orgasm and detumescence. **Libido or sexual desire**: Libido is defined as the biological need for sexual activity (the sex drive) and frequently is expressed as sex-seeking behavior. Its intensity is variable between individuals as well as within an individual's over a given time. Higher serum testosterone appears to be associated with greater activity in healthy older but not younger men.
- **Erection**: Erection is the enlarged and rigid state of the sexually aroused penis sufficient enough for vaginal penetration. It results from multiple psychological and sensory stimuli arising from imaginative, visual, auditory, olfactory, gustatory, tactile and genital reflexogenicsources.
- **Ejaculation**: Ejaculation is the act of ejecting semen. It is a reflex action that occurs as a result of sexual stimulation. It is made up of two sequential processes. The first process called emission is associated with deposition of seminal fluid into the posterior urethra while the second process is the true ejaculation, while is the expulsion of seminal fluid from the posteriorurethra through the penile meatus.
- **Orgasm**: This is the climax of sexual excitement. The entire period of emission and ejaculation is known as the male orgasm.
- **Detumescence**: This is the subsidence of an erect penis after ejaculation.

2.2. Physiology of Normal Penile Erection [3,4]

A normal penile erection is the final common pathway of the integrative synchronized action of psychological, neuronal, hormonal, vascular and cavernous smooth muscle systems. The patient must also be psychologically receptive to sexual stimuli. Any abnormality involving these systems, whether from medication or disease, has a significant impact on ability to develop and sustain an erection, ejaculate and experience orgasm. Tumescence, the vascular filling of the cavernous bodies, relies on neural and hormonal mechanisms operating at various level of the neural axis. This is unique among visceral functions because it requires central neurological input.

The penis comprises two corpora cavernosa on dorsal and one corpus spongiosum on ventral side. The corpus spongiosum surrounds the urethra and forms the glans penis. The corpora are composed of multiple, interconnected sinuses, which can fill with blood to produce an erection. The corpora are encased by the tunica albuginea, a fibrous tissue membrane, which has limited distensibility. In the flaccid state, arterial flow into and venous outflow from the corpora are balanced. During the erectile phase, arterial blood flow increases and blood fills the sinusoids within the corpora, which cause penile swelling and elongation. The erection is prolonged by subtunical venules by the swollen corpora.

Arterial flow into the corpora is enhanced by acetylcholine mediated vasodilatation. Acetylcholine does not directly enhance arterial flow to the corpora or increase sinusoidal filling to the corporal tissue. Rather, acetylcholine is a co-neurotransmitter, which works along with other nonpeptidnergic intracellular neurotransmitter including cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP), or vasoactive intestinal polypeptide to produce vasodilatation.

Acetylcholine probably works through two different ways to produce an erection. In one pathway, Ach, in presence of sexual stimulation to genital tissue, enhances the production of nitric acid by endothelial cells and noradrenergic and nor cholinergic (NANC) neurons .Nitric acid enhances the activity of guanylate cyclase enzyme, which increases the conversion of cyclic guanosine triphosphate to cGMP. cGMP decreases intracellular calcium conc. in smooth muscle cells of penile arteries and cavernosal sinuses. As a result, smooth muscle relaxation occurs, which enhances arterial blood flow to and filling of corpora. An erection occurs in alternative pathway, acetylcholine stimulates a smooth muscle cell membrane receptor to enhance the activity of adenyl cyclase. Adenyl cyclase increases the conversion of cyclic adenosine triphosphate (cATP) to cyclic adenosine monophosphate (cAMP) a potent muscle relaxant. Similar to cGMP, cAMP decreases intracellular calcium conc. to produce smooth muscle relaxation in cells of arteries and cavernosal sinuses. Arterial blood flow to and blood filling of corpora are enhanced and penile erection results.

2.3. Sexual Dysfunction[5]

Sexual dysfunctions cover a wide variety of problems, including erectile dysfunction (ED) and premature or delayed ejaculation in men, spasms of the vagina, pain with sexual intercourse, and problems with sexual desire (libido) and response. Men over age 65 are at higher risk for ED, although ED is not a normal part of aging. Decreased sexual desire is the most common complaint among women, affecting up to 43% of women. The problem may be psychological, physical, or a combination of both. Sexual dysfunction or sexual malfunction refers to a difficulty experienced by an individual or a couple during any stage of a normal sexual activity, including desire, arousal or orgasm. To maximize the benefits of medications and behavioral techniques in the management of sexual dysfunction it is important to have a comprehensive approach to the problem. A thorough sexual history and assessment of general health and other sexual problems (if any) are very important. Assessing (performance) anxiety, guilt (associated with masturbation in many Indian men), stress and worry are integral to the optimal management of sexual dysfunction. When a sexual problem is managed inappropriately or sub-optimally, it is very likely that the condition will subside immediately but re-emerge after a while. When this cycle continues, it strongly reinforces failure that eventually makes clients not to access any help and suffer it all their life. So, it is important to get a thorough assessment from professionals and therapists who are qualified to manage sexual problems. Internet-based information is good for gaining knowledge about sexual functioning and sexual problem but not for self- diagnosis and/or self-management.

III. Aphrodisiacs Agents [6]

An **aphrodisiac** is a substance that increases sexual desire. The name comes from Aphrodite, the Greek goddess of sexuality and love. Throughout history, many foods, drinks, and behaviors have had a reputation for making sex more attainable and/or pleasurable. However, from a historical and scientific standpoint, the alleged results may have been mainly due to mere belief by their users that they would be effective (i.e., the placebo effect). In particular, Western medical science has no substantiated claims that any particular food increases sexual desire or performance. Some purported aphrodisiacs gain their reputation from the principles of sympathetic magic, for example oysters, due to their shape. The same factor explains the trade in the phallic- looking horn of the rhinoceros. Other animal-based aphrodisiacs gain their reputation from the apparent virility or aggressiveness of the animal source, such as tiger penis.

Knowing what is an aphrodisiac is important; aphrodisiac: it is a substance or activity which is supposed to heighten sexual interest and desire. There are currently many substances throughout history have been used as aphrodisiacs, and some cultures have built up their own aphrodisiac rituals such as dances which purpose is to highlight female beauty arousing the audience. The efficacy of various products that are currently included in the list of aphrodisiacs is a subject of debate, for the reason that scientific studies have been performed on these substances and on top of that there is a greater concern taking into consideration the fact that some aphrodisiacs, such as rhinoceros horn, are putting endangered animals at risk of extinction. So, besides knowing what is an-aphrodisiac you have to make the right choices.

So after knowing what is an aphrodisiac you have to know that the term comes from Aphrodite, the Greek goddess of sensuality and love, and so the Greeks referred to sexual pleasure as aphrodisiac, and that is why it stands to reason that a substance which enhances this experience would be known as an aphrodisiac? Many foods are part of the list of aphrodisiacs, including some surprising foods like arugula, garlic, mustard, and asparagus and in some cultures the consumption of specific herbs is supposed to enhance sexual desire, and many societies also include animal products in the list of aphrodisiacs. Tiger penis and rhinoceros horn, for example, are part of the list of aphrodisiacs because these animals are virile and strong and so people want to get those qualities out of them by using their products.

IV. Plant Profile [7,8]

4.1. Scientific name: *Euphorbia hirta.Linn.*

4.2.Family: *Euphorbiaceae.*

4.3. Vernacular name: -Dugdhika, Mothi dudhi, Dudhi.

4.4. Description: - An erect herb up to 50 cm tall with greenish yellow OR white flowers, stem covered with Yellowish hairs. Leaves are in opposite pairs. *Euphorbia hirta.Linn.* is a small weed, erect or prostrate. It is hairy and greenish to reddish in color. It exudes a white latex as soon as it is cut. The plant has a deep root system. The short main stem branches quickly to give secondary creeping stems. These stems are abundantly hairy and often tinged with red. Leaves, with short petiole, are arranged in pairs along the stem. Their edge is serrated. They are hairy on both sides. The flowers are grouped into balls, alternately arranged along the stem. They are greenish, contained in a small cup with four glands of white edge. A globular fruit with 3 quarters emerge from the cut.

The leaves are simple and distichious opposite, short-stalked. They measure up to 5 cm long and 2 cm wide. The bases of petioles are joined by a thin stipular collar with 2 to 4 filiform tines. Lamina is oval to elliptical, dark green in colour. Base of lamina highly asymmetric, vertex apex. Finely serrated margin. Upper and lower pubescent (denser hairs along the ribs on the underside, more scattered on the upper lamina).

Fig- 5. Leafs of *Euphorbia hirta*. Linn. **Fig-6. Plant of *Euphorbia hirta*. Linn.**

4.5. Plant part used: - Leaves,whole plant.
4.6. Chemical constituents: - Alkaloids, Saponins, Tannins, Steroids and Flavonoids etc.
4.7. Traditional uses: -Used in treatment of cough, asthma, diarrhea, piles and semen debility, worm infection, leprosy, skin diseases, urinary infection and dis-urea. It is aphrodisiacand enriches blood etc.[7]

V. Methods and Materials

5.1.*Chemicals:*
All the chemicals were of analytical grade and were obtained from Merck India Limited and Loba Chemie Limited, India

5.2.Authentication of plant material:
The fresh leaves of *E. hirta Linn.* plant was collected from local area of Nagpur region. The plant specimen was dried and their herbarium sheet was prepared .*E. hirta* was botanically identified & authenticated by Dr.Alka Chaturvedi ,Professor & Head,Department of Botany,under the R.T.M. Nagpur University, Nagpur in July 2010.

Fig-7:Hebarium of *Euphorbia hirta.* Linn.plant

5.3. Plant material collection:

The leaves were separated from the whole plants and collected. The leaves were initially washed with distilled water to remove debris and dust particles, dried on paper towels at room temperature (37±1)0 C. The leaves were avoided from exposure to sunlight to prevent the loss of active components. Dried leaves were packed in polyethylene bags. In the laboratory, after drying, the leaves were subjected to size reduction in a mechanical grinder, followed by a blender, to get coarse or fine powder. The coarse powder was then passed through sieve no. 40 mm to get desired particle size and stored in a well – closed plastic containers at room temperature till required for use.

VI. Determination of Physio-Chemical Constant: [14,15,16,17]

Physico-chemical values such as the ash values, extractive values and percentage of loss on drying were carried out as per the standard method described in Indian Pharmacopoeia (1996).

6.1. Ash value:

6.1.a.*Determination of Ash values:* Crude drugs quality and purity was determined by ash values. Sodium, potassium, magnesium, carbonates of calcium salts, phosphates and silicates constitutes the total ash values. Powdered plant materials were subjected to total ash, acid insoluble ash and water soluble ash

6.1.b.*Determination of Total Ash:* Coarsely powdered leaf material of about 2 to 3gms was weighed and transferred to a pre-weighed silica crucible. In the crucible, powdered drug was spread evenly as fine layer and incinerated by gradually increasing the temperature not exceeding 4500 c until colourless, indicates the carbon free. After incineration completion, silica crucible was cooled in a desiccator and weighed. The same procedure was repeated to get a constant weight. The percentage of total ash was determined with reference to the shade dried plant material.

6.1.c.*Acid –insoluble ash:* The ash obtained from the above method was boiled for 5-10min with 25ml of dilute HCl. The insoluble ash was collected in an ashless filter paper or a Gooch crucible. The crucible or filter paper was washed with hot water. The acid insoluble ash was transferred to a pre weighed silica crucible. This was repeated until constant weight obtained. The acid insoluble ash percentage was calculated with reference to the air –dried drug.

6.1.d.*Water soluble ash value:* The ash obtained in the determination of total ash was boiled for about 5- 10min in 25ml of water. The insoluble matter was collected in a silica crucible, washed with hot water. The insoluble ash so obtained was transferred to a pre-weighed silica crucible. The silica crucible was heated gradually to a temperature of 4500 c for 15 min to get a constant weight. The crucible was cooled and weighed. The percentage of water soluble ash was calculated by subtracting weight of insoluble matter from the weight of total ash.

Table: 1. Ash value

1	**Total ash value**	**8.4%**	**Not more 12 %**
2	**Water soluble ash value**	**1.06%**	**Not more than 10 %**
3	**Acid insoluble ash value**	**1.1%**	**Not more 07 %**

6.2. Moisture content (loss on drying) (LOD*):* 2gms of powder drug was accurately weighed and taken in a porcelain dish. The dish was kept in a hot air oven maintained at a temperature 110 c for 4 hours until a constant stable weight was recorded. The procedure was repeated. The dish was cooled in a desiccator at room temperature and weighed.

Table:2.Moisture content

1	**Moisture content:**	**7.85 %**

VII. Microscopy: [10,14,17,18]

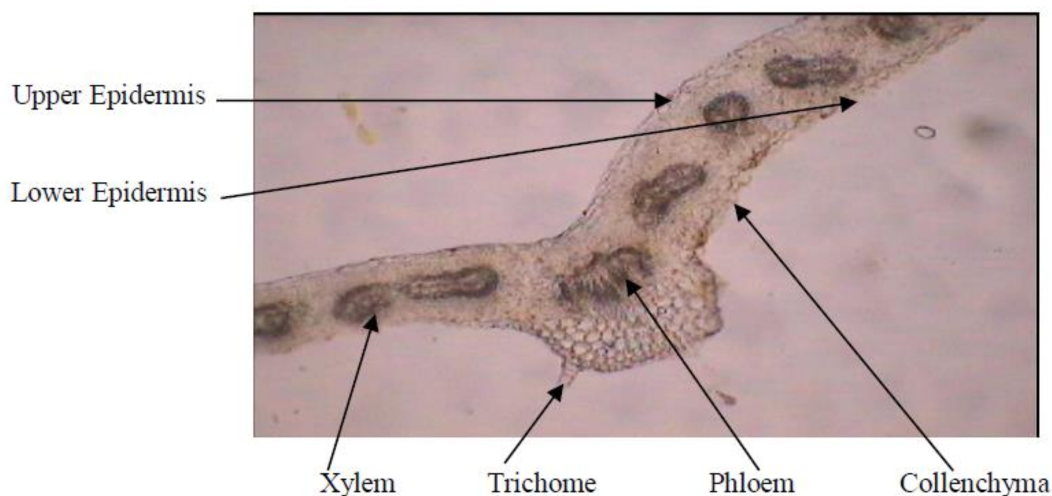
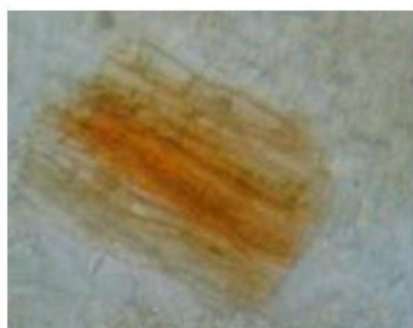


Fig-8.Transverse section of *Euphorbia hirta Linn.*leaf.

VII.1.Powder Microscopy of *Euphorbia hirta Linn*.leaf



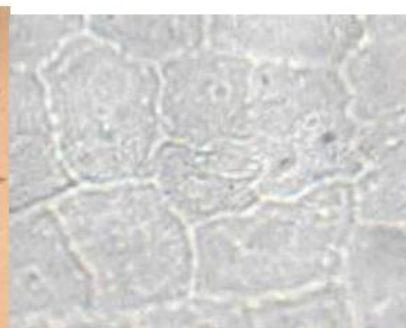
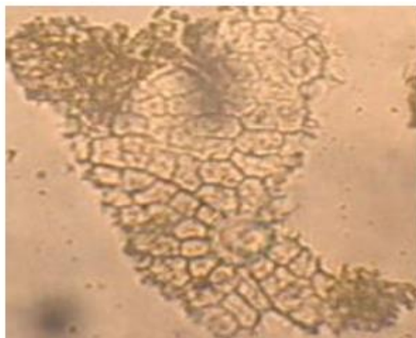
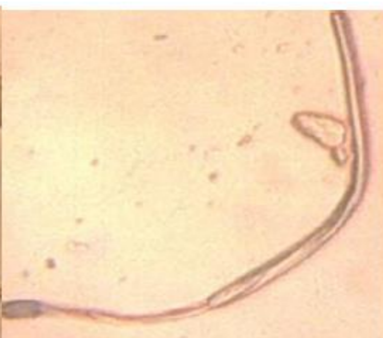


Fig-9. Lignified Xylem **Fig-10. Pericyclic fibre** **Fig-11.Anomocytic stomata**





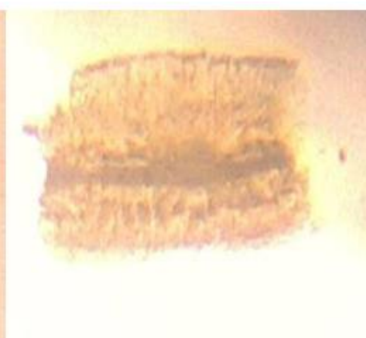


Fig-12.Epidermal cells with stomata **Fig-13.Fibre** **Fig-14.Scalifarm vessel**

VIII.Extraction & Fractionation[19]

8.1.Principle: To prepare various extracts of *Euphorbia hirta Linn*., a successive solvent extraction procedure was adapted. The plant materials were subjected to successive extraction with different solvents, starting from solvent of low polarity to high polarity.

Materials: Dried leaves powder of *Euphorbia hirta Linn.*
Apparatus: Soxhlet apparatus
Solvents: Petroleum ether, hydro alcohol
Procedure: An about 200 gm of powdered drug of the selected medicinal plant was subjected to extraction defatted by using Petroleum ether & then this defatted material recharged with hydroalcohol as solvent in a Soxhlet apparatus. The extraction was continued until the solvent in the timble becomes clear or colourless. Then the heating was stopped and the mixture from distillation flask was collected and cooled. Then this mixture was filtered and concentrated by using evaporator at room temperature. The extract was dried at room temperature and stored in amber coloured glass jar in a freezer or desiccator and was used for further experiments [20]

8.2.Fractionation: The hydro alcoholic extract then fractionated by using separating funnel with ethyl acetate soluble extract & acetone soluble extract.

8.3.Determination of extractive value*:* Extractive values are used to evaluate the nature of phytochemicals present in the crude drugs. The extractive yield is a measure of the solvent's efficiency to extract specific components from the original material and it was defined as the amount of extract recovered in mgs compared with the initial amount of whole plant. It is presented in percentage and was determined for each techniques tested.

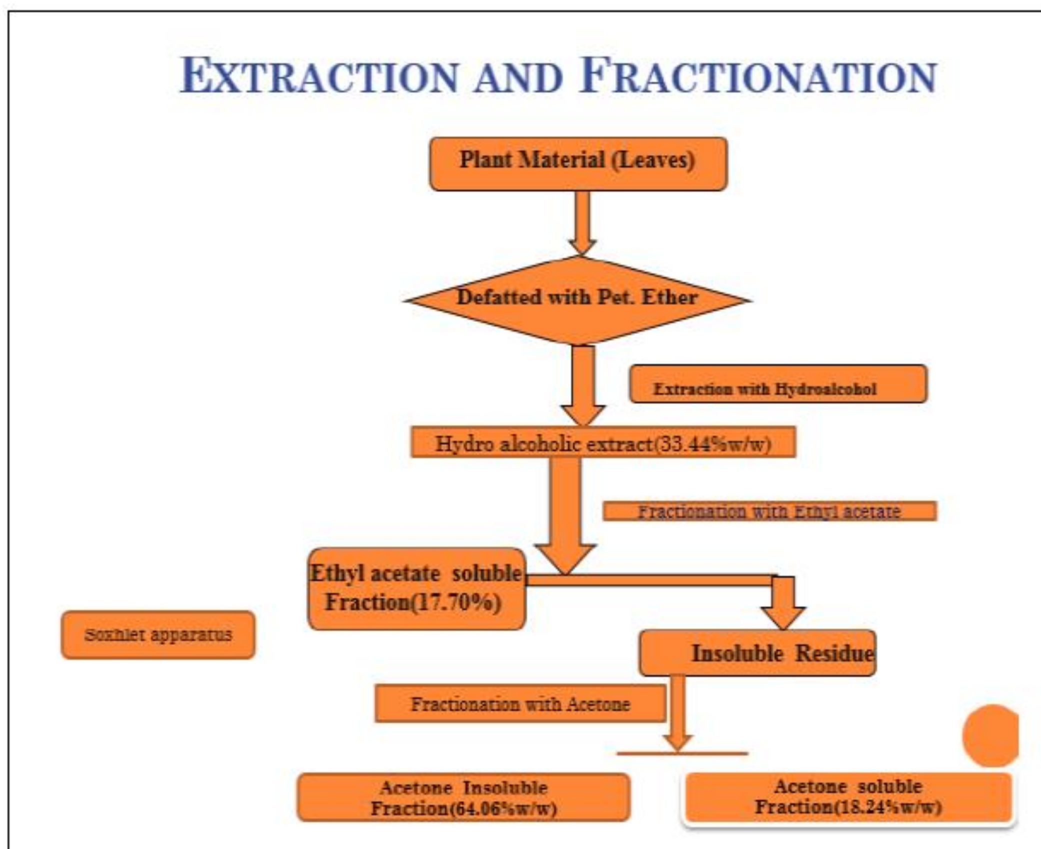


Fig-15.Extraction & fractionation of *Euphorbia hirta Linn.*leaf extract.

Table:3.Extractive values

1	**Water soluble extractive value**	**22.16%w/w Not less 10%w/w**
2	**Alcohol soluble extractive value**	**14..08%w/w Not less 3 %w/w**

IX.Qualitative Phytochemical Screening of *Euphorbia hirta Linn.*leaf extracts [14, 15, 16]

Hydro alcoholic extract obtained by successive solvent extraction method and ethyl acetate & acetone extract by successive fractionation method. The above three concentrated extracts of the *Euphorbia hirta Linn* were subjected to various standard phytochemical tests procedures to detect the presence or absence of various active phytoconstituents present in the crude extracts

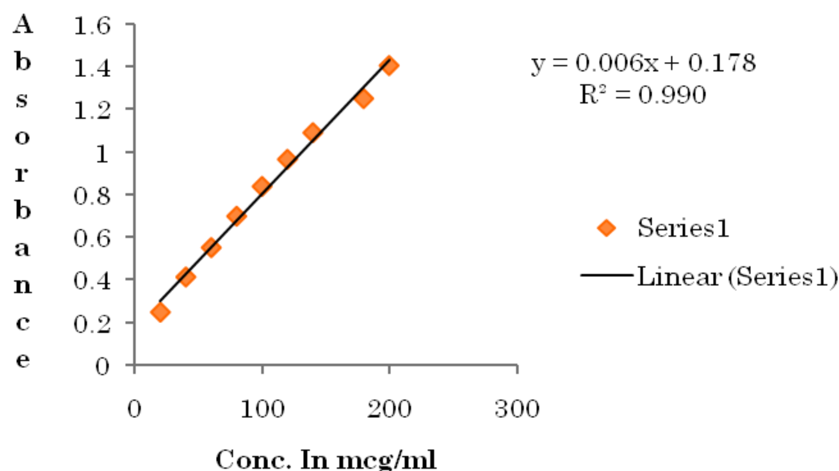
Table:4.Qualitative Phytochemical Screening of *Euphorbia hirta Linn.*leaf Extracts

Chemical Constituent	Hydro alcoholic (HA)	Ethyl acetate soluble fraction of HA(EAS)	Acetone soluble fraction of HA(ACS)	Acetone insoluble residue of HA(ACIS)
Proteins & amino acids	–	–	–	–
Carbohydrates	+	–	–	+
Steroids	+	+	–	–
Saponins	+	+	–	–
Tannins & Phenolics	+	+	+	–
Flavanoids	+	+	+	–
Alkaloids	–	–	–	–

+ Presence of compounds, - Absence of compounds.

X.Determination of Total Phenolic Content of *Euphorbia hirta Linn.*leaf Extracts[20, 21, 22]

The total phenols content of the extract was determined by the Folin–Ciocalteu method The concentration gradient of gallic acid was prepared as standard solution (0–50 µg/mL), and the calibration curve was established using gallic acid. The samples were diluted with DMSO as sample solutions, after they were made up to 10 mL by methanol. The 6 mL of distilled water and 100 µL of sample or standard solution and 0.5 mL Folin–Ciocalteu reagent were mixed for 5 min, followed by the addition of 1.5 mL of 20% sodium carbonate and 1.9 mL of distilled water. The mixture was placed for 120 min at room temperature. Every experiment was performed in triplicate. The absorbance of the mixture was measured at 758 nm using a UV spectrophotometer. Total phenols content of the samples was expressed as mg of gallic acid equivalents (GAE) per gram dried extract. The experimental results are expressed as mean ± standard error of mean (SEM) of three replicates.



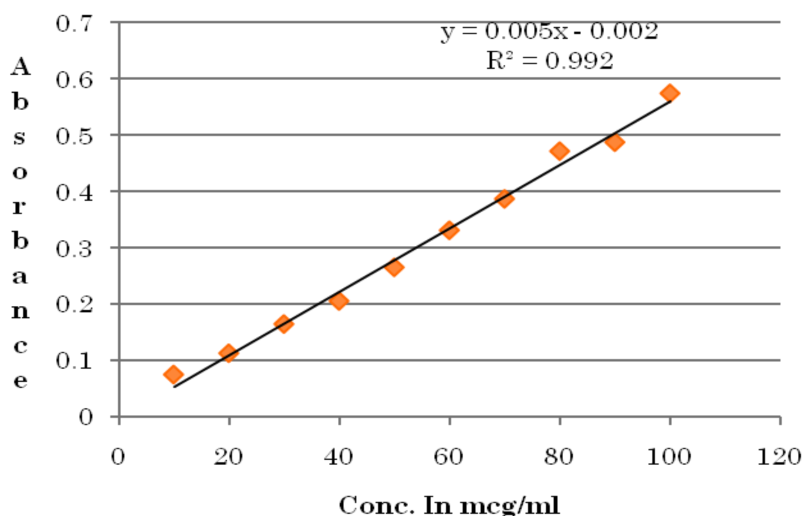
Graph:1.Absorbance vs Conc.Total Phenolic Content of *Euphorbia hirta Linn.*leaf extracts

Table:5.Total Phenolic Content of *Euphorbia hirta Linn.*leaf extracts

Plant part	Types of extracts /fractions	Total polyphenol (Gallic acid equivalent mg/g)				Avg. (mg/gm)
Leaves	Hydro alcoholic extract(HA)	0.1 mg/ml	0.3 mg/ml	0.5 mg/ml	1.0 mg/ml	119.60
		11.10	34.08	49.16	155.5	
	Ethyl acetate fraction(EAS)	12.58	37.83	64.16	183.50	140.89
	Acetone fraction(ACS)	9.83	32.66	44.83	140.00	109.17

XI.Determination of Total Flavonoid Content (TFC) [23, 24, 25, 26]

Total flavonoid content was determined by aluminum chloride colorimetric assay. The concentration gradient of quercetin was prepared as standard solution (0–80 µg/mL), and the calibration curve was established using quercetin. The samples were diluted with DMSO as sample solutions, after they were made up to 50 mL by methanol. The 4 mL of distilled water and 1 mL of sample or standard solution, and 300 µL $NaNO_2$ (5%) were added to a glass cuvette. Three hundred microliters of $AlCl_3$ (10%) were added after 5 min and 2 mL NaOH (1 M) and 2.4 mL distilled water were added after 6 min. The mixture was placed for 10 min at room temperature. Every experiment was performed in triplicate. The absorbance of the mixture was measured at 510 nm using a UV spectrophotometer. Total flavonoid content of the samples was expressed as mg of quercetin equivalents (QE) per gram dry extract. The experimental results are expressed as mean ± standard error of mean (SEM) of three replicates.



Graph:2.Absorbance vs Conc.Total Flavanoid Content of *Euphorbia hirta Linn.*leaf extracts

	Types of extracts /fractions	Total Flavonoids content (Quercetin equivalent mg/g)				Avg. (mg/gm)
Leaves	Hydro alcoholic Extract(HA)	0.1mg/ml	0.3mg/ml	0.5mg/ml	1.0mg/ml	mg./gm.
		11.2	30.00	49.5	107.4	104.57
	Ethyl acetate fraction of HA(EAS)	14.0	57.2	110.2	227.8	194.66
	Acetone fraction of HA(ACS)	7.5	24.2	41.00	83.2	80.19

Table:6.Total Flavanoid Content of *Euphorbia hirta Linn.*leaf extracts

XII.Antioxidant Activity [27,28 ,29, 30, 31]

12.1.DPPH radical scavenging assay

DPPH (1-diphenyl-2-picrylhydrazyl) is a stable free radical that has a maximum absorption of 515–517 nm (purple color). When an antioxidant (as a hydrogen donor) reacts with DPPH, DPPH is reduced to DPPH-H, and as a consequence the solution turns from purple to yellow. Therefore, the antioxidant activity of DPPH samples can be evaluated by the decrease in absorption at 515–517 nm. The potential scavenging abilities of the samples were assessed using ascorbic acid (Merck, Germany) as positive control [38].

Briefly, 0.5 mL of various concentrations of each sample (10, 50, 100, 250, 500, 750 and 1000 μg/mL) or ascorbic acid (0.5, 0.25, 0.1, 0.05, and 0.01 mM) was added into a tube containing 0.5 mL of 0.6 mM DPPH solution dissolved in methanol and the volume was made uniformly to 4 mL using methanol. The solution was mixed and then allowed to stand in the dark at room temperature for 30 min. Absorbance was taken at 515 nm using methanol as blank on UV-visible spectrometer. Then, 0.5 mL of DPPH was added to 3.5 mL of methanol and absorbance was taken for control reading. All analyses were run in triplicate [39].

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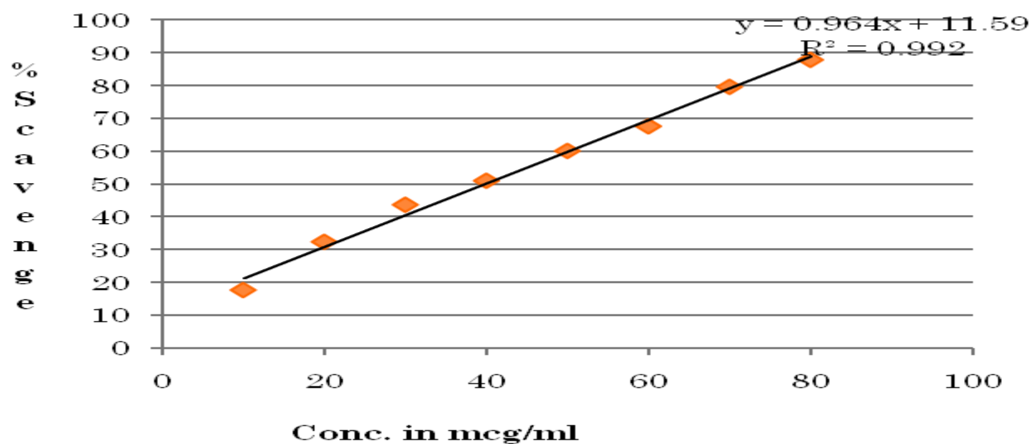
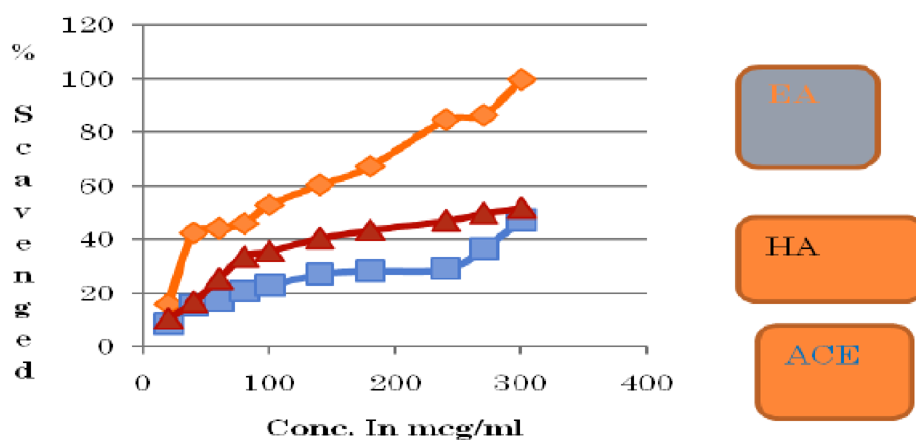


Graph:3. % Scavenge vs. Conc.Antioxidant Content of *Euphorbia hirta Linn.*leaf extracts



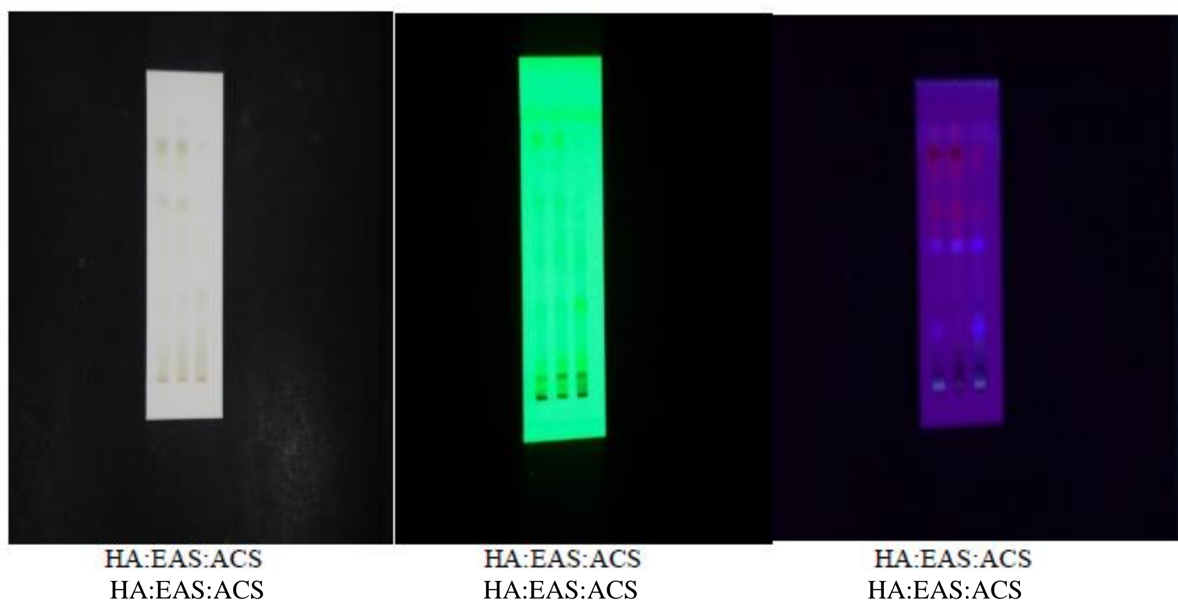
Graph:4. % Scavenge vs. Conc.Antioxidant Content of *Euphorbia hirta Linn.*leaf extracts

Table:7. Antioxidant content of *Euphorbia hirta Linn.*leaf extracts.

	Types of Extracts /fractions	DPPH IC_{50} value (µg/ml)
Standards	**Ascorbic acid**	38.99
***E.hirta* Leaves**	**HA Extract**	261.83
	EAS Fraction	95.05
	ACS Fraction	318.40

XIII.TLC Study: [32, 33]

13.1.Comparative TLC images of HA, EAS and ACS Extracts At Visual, 254 and 366



HA:EAS:ACS HA:EAS:ACS HA:EAS:ACS

13.2.*E.hirta* Hydro alcoholic extract
Scanning wavelength: 254nm
Stationary phase: HPTLC precoated, silica gel G 60 F_{25}(Merck, Germany)
Mobile phase : Toulene:Ethyl acetate:Methanol:Formic acid(6:3.5:0.5:0.1)

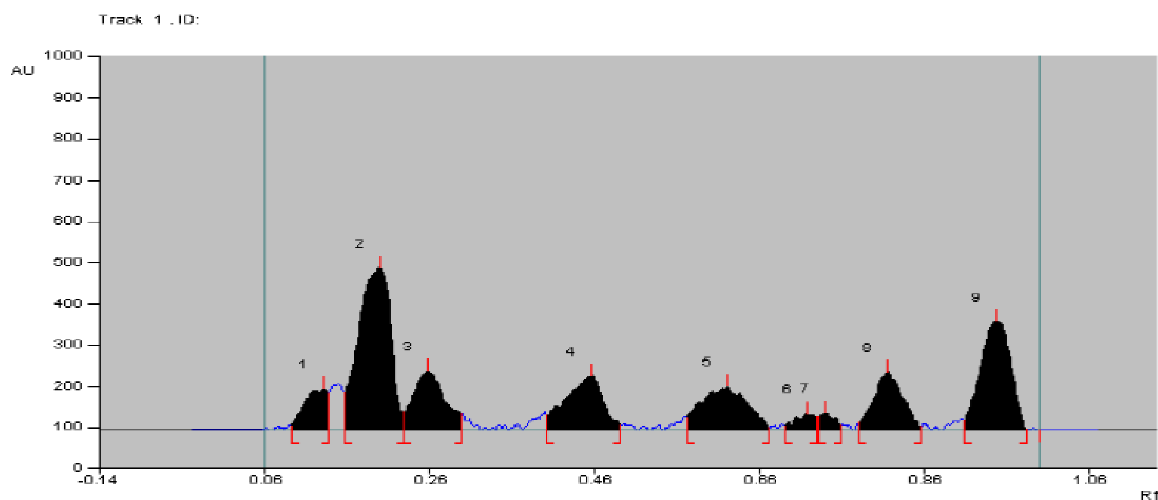


Fig-17. 9 spots were found with the Rf values 0.13,0.20,0.26,0.46,0.62,0.72,0.74,0.82,0.95.

13.3.*E.hirta* Hydro alcoholic extract
Scanning wavelength: 366nm
Stationary phase: HPTLC precoated, silica gel G 60 F_{25}(Merck, Germany)
Mobile phase: Toulene: Ethyl acetate: Methanol: Formic acid(6:3.5:0.5:0.1)

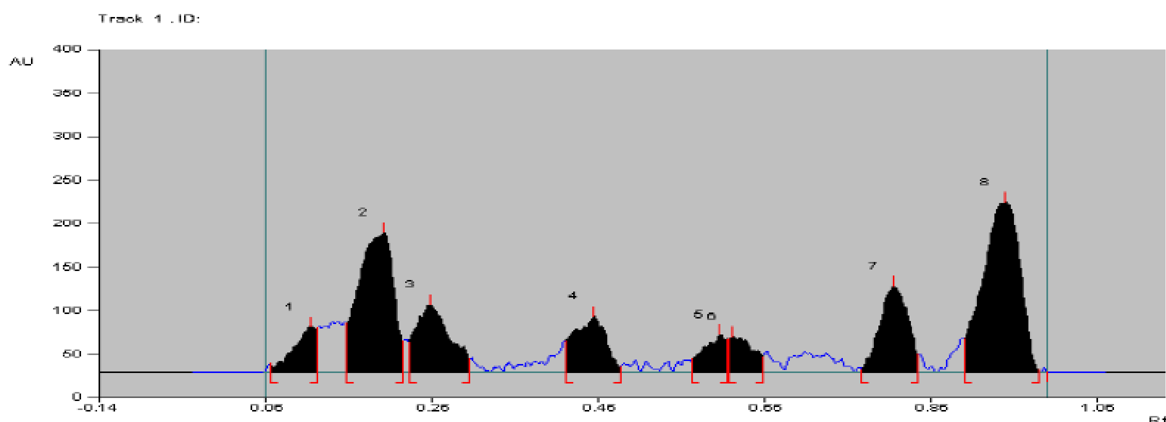


Fig-18. 8 spots were found with the Rf values 0.11,0.20,0.26,0.45,0.61,0.62,0.82,0.95.

13.4.*E.hirta* Ethyl acetate fraction
Scanning wavelength: 254nm
Stationary phase: HPTLC precoated, silica gel G 60 F_{25}(Merck, Germany)
Mobile phase : Toulene:Ethyl acetate:Methanol:Formic acid(6:3.5:0.5:0.1)

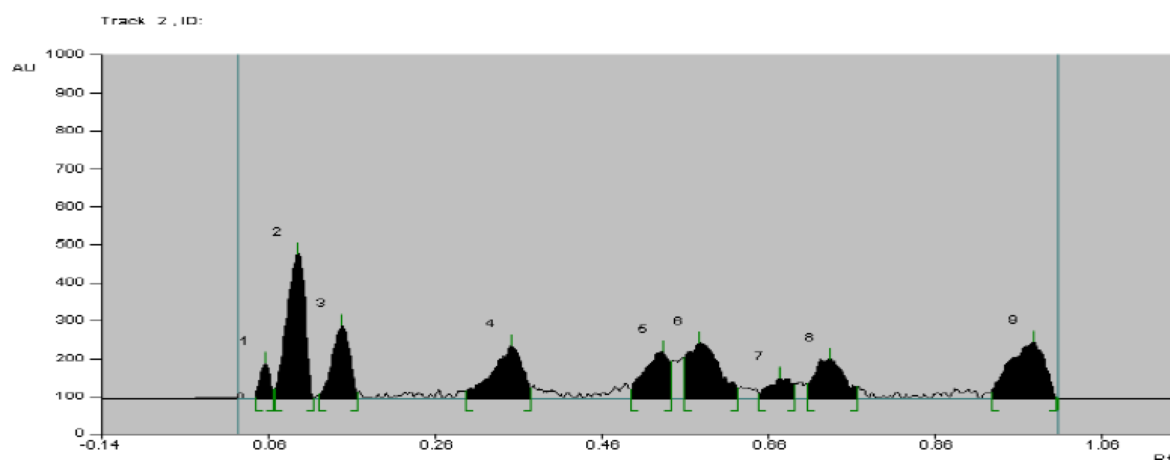


Fig-19. 9 spots were found with the Rf values 0.06,0.09,0.15,0.35,0.53,0.58,0.67,0.73,0.98.

13.5.*E.hirta* . Ethyl acetate fraction
Scanning wavelength: 366 nm
Stationary phase: HPTLC precoated, silica gel G 60 F_{25}(Merck, Germany)
Mobile phase : Toulene:Ethyl acetate:Methanol:Formic acid(6:3.5:0.5:0.1)

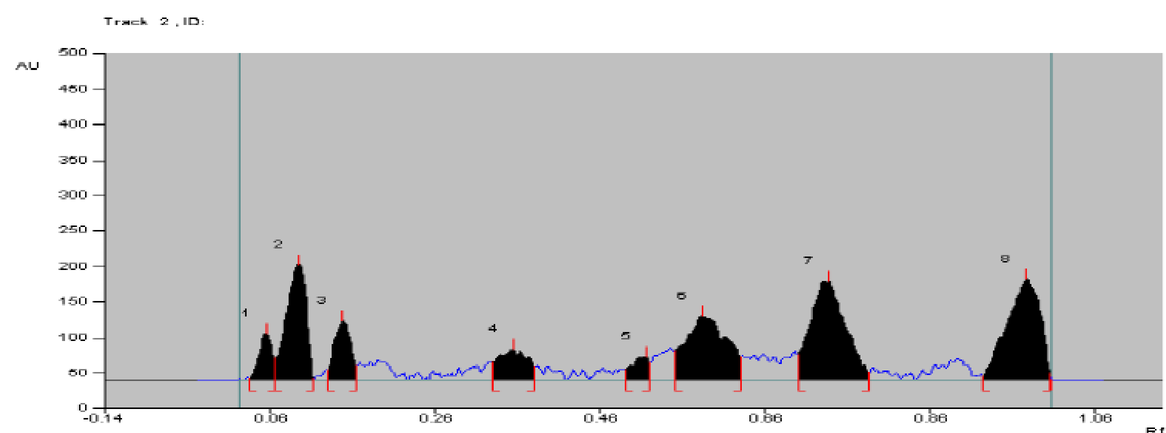


Fig-20. 8 spots were found with the Rf values 0.05,0.09,0.15,0.35,0.52,0.58,0.74,0.98.

***E.hirta* . Acetone fraction**
Scanning wavelength: 254 nm
Stationary phase : HPTLC precoated, silica gel G 60 F_{25}(Merck, Germany)
Mobile phase : Toulene:Ethyl acetate:Methanol:Formic acid(6:3.5:0.5:0.1)

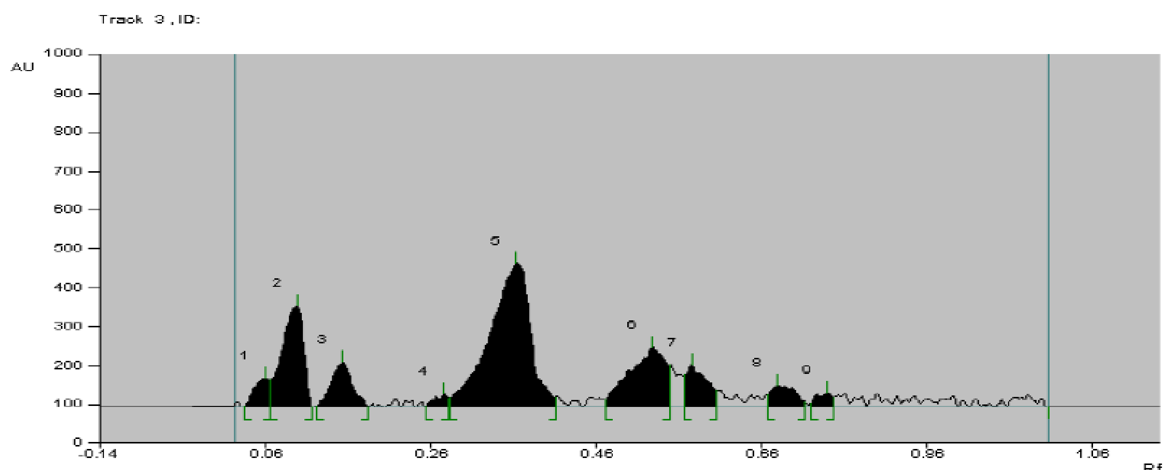


Fig-21. 9 spots were found with the Rf values 0.06,0.10,0.15,0.28,0.36,0.53,0.58,0.68,0.74

***E.hirta* . Acetone fraction**
Scanning wavelength: 366 nm
Stationary phase: HPTLC precoated, silica gel G 60 F_{25}(Merck, Germany)
Mobile phase : Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)

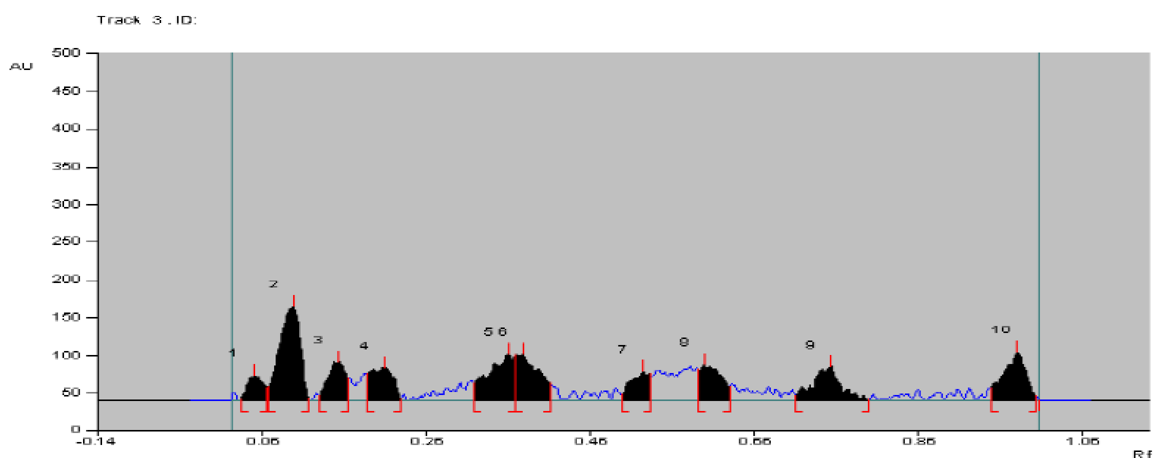


Fig-22. 10 spots were found with the Rf values 0.05,0.10,0.15,0.21,0.36,0.38,0.52,0.60,0.75,0.98

Table:8.HPTLC of *Euphorbia hirta Linn.*leaf Extracts.

Test extract	Solvent system	Number of Spots
Hydro alcoholic extract (HA)	Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)	09
Ethyl acetate Fraction of HA (EAS)	Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)	09
Acetone Fraction of HA (ACS)	Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)	08

XIV.Aphrodisiac Activity Of *Euphorbia hirta* Leaves [34, 35, 36]

14.1.Animals

Fourteen week old, sexually mature male and female albino rats were used for the study. All animals were housed in polypropylene cages and maintained in a 12h:12hr light:dark cycle, at ambient temperature with water and solid pellet food (Epol-SA) ad libitum. Ethical care and handling of experimental animals was observed at all times and the study was approved by Animal ethical committee of University Dept.of Pharmaceutical Sciences RTM Nagpur University ,Nagpur Prior to commencement of the study, male rats were exposed to receptive females once a week for 2 consecutive weeks and those males showing sexual activity within 5 min after exposure to the female were selected for the study. The sexually active animals were randomly divided into 5 oral treatment experimental groups each consisting of 6 rats. The groups were: Control group (distilled water), Hydroalcoholic extarct (300 mg/kg body weight; positive control) ethyl acetate fraction of hydroalcoholic extract (200 mg/kg body weight) Acetone fraction of hydroalcoholic extract (300 mg/kg body weight) & L-DOPA (100 mg/kg body weight) as standard was used as the positive control and a dose of 300mg/kg body weight was selected based on published reports (Watcho et al., 2004, 2007a; Quasie et al., 2008).

14.2.Sexual behavior tests

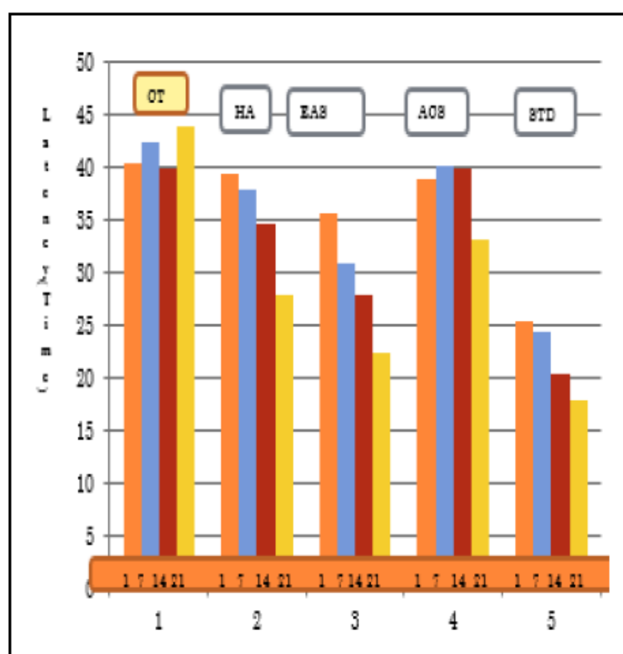
Sexual behaviour tests were carried out using standard methods (Yakubu et al., 2007; Watcho et al., 2007a). An acute sexual behaviour observation was carried out 2 hours after a single dose of plant extract (acute effect) then on days 14 and 28 of daily treatment of male rats. In these tests, female rats artificially brought to oestrus by treatment with estradiol (500 µg/kg s.c., 48h before tests) and progesterone (5.0 mg/kg s.c., 4h before tests) were introduced into the observation cage of the male animal with 1 female to 1 male ratio. The observation for mating behavior was started immediately after introduction of the female and parameters were recorded as they occurred for 20 minutes. The parameters of male sexual behavior that were monitored were: mount latency (ML= time interval between the introduction of the female and the first mount by the male); intromission latency (IL = time interval from the introduction of the female to the first intromission by the male); mount frequency (MF = number of mounts without intromission from the time of introduction of the female); intromission frequency (IF= number of intromissions from the time of introduction of the female); ejaculation frequency (EF= number of ejaculations from the time of introduction of the female). Copulatory efficiency percent (%) was calculated as IF/MF*100.

Table:9.Dosing of extracts, control & standard [37, 38, 39, 40, 41, 42]

Groups	Dose	No. of Animals	Route
Control	Dist. water 1 ml	06	Oral
L-dopa	100mg/kg	06	Oral
Hydro alcoholic extract	300mg/kg	06	Oral
Ethyl acetate fraction	300mg/kg	06	Oral
Acetone Fraction	300mg/kg	06	Oral

VI.Results

Mounting Latency, Mounting Frequency, Intromission Latency ,Intromission Frequency, Ejaculation Latency, Ejaculation Frequency, Post EjaculationLatency

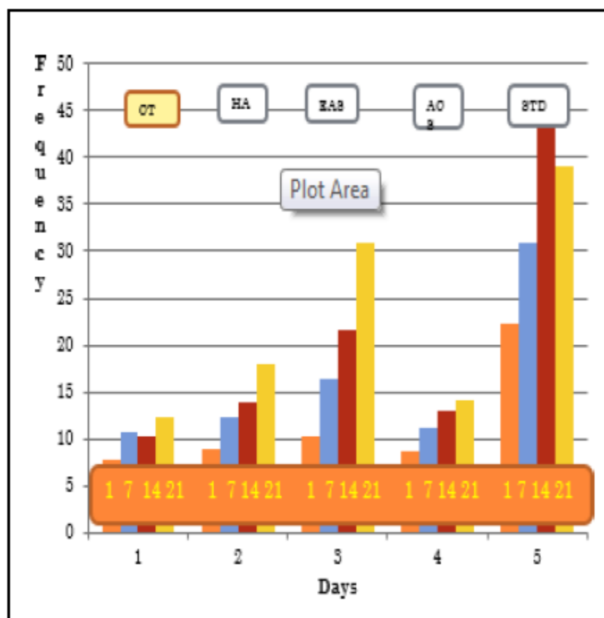


Graph:5.Mounting LatencyCT, HA, EAS, ACS and STD

Mounting Latency

Group	0 day	7day	14day	21 day
CT	40.33±0.88	42.33±1.45	40.00±1.52	44.00±1.52
HA	39.33±0.67	38.00±0.58	34.67±0.88	28.00±0.33
EAS	35.67±0.33	31.00±0.58	28.00±1.20	22.33±1.15
ACS	39.01±1.57	40.21±0.57	40.00±1.67	33.20±0.33
STD	25.33±2.33	24.33±2.03	20.33±0.88	18.00±1.88

Table:10.Mounting Latency CT, HA, EAS, ACS and STD

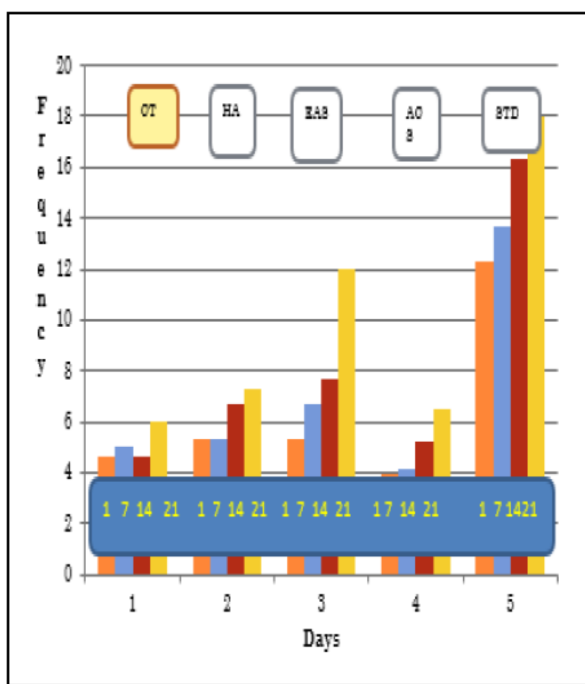


Mounting Frequency

Group	0 day	7day	14day	21 day
CT	40.33±0.88	42.33±1.45	40.00±1.52	44.00±1.52
HA	39.33±0.67	38.00±0.58	34.67±0.88	28.00±0.33
EAS	35.67±0.33	31.00±0.58	28.00±1.20	22.33±1.15
ACS	39.01±1.57	40.21±0.57	40.00±1.67	33.20±0.33
STD	25.33±2.33	24.33±2.03	20.33±0.88	18.00±1.88

Graph:6.Mounting Frequency CT, HA, EAS, ACS, STD

Table:11.Mounting Frequency CT, HA, EAS, ACS, STD

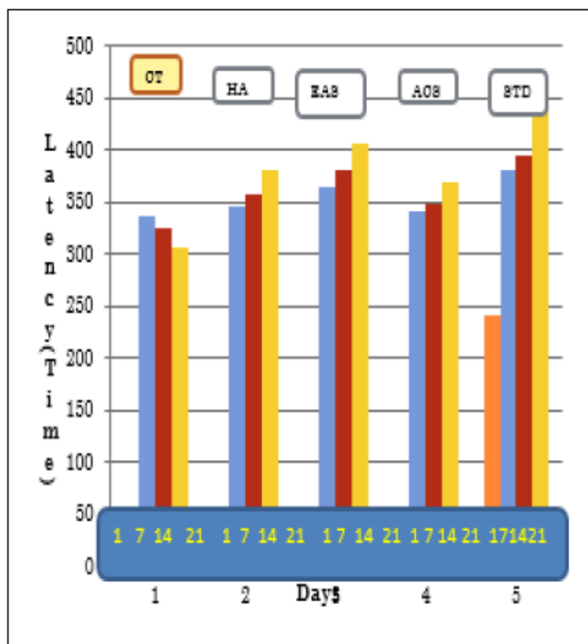


Intromission Frequency

Group	0 day	7day	14day	21 day
CT	4.67±0.33	5.00±0.58	4,66±0.88	6.00±0.21
HA	5.33±0.33	5.33±0.88	6.67±0.33	7.25±0.67
EAS	5.33±0.68	6.67±0.89	7.67±0.33	12.00±0.67
ACS	4.00±0.33	4.12±0.57	5.21±0.58	6.54±0.67
STD	12.33±2.33	13.67±0.67	16.33±0.88	18.00±0.33

Graph:7. showing Intromission Frequency CT, HA, EAS, ACS, STD

Table:12. Intromission Frequency CT, HA, EAS, ACS, STD

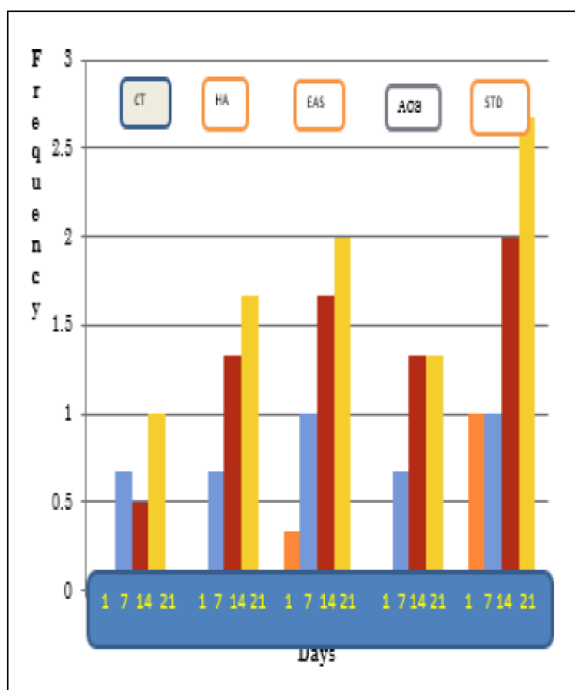


Graph:8. showing EjaculationLatency CT, HA, EAS, ACS, STD

Ejculation Latency

Group	0 day	7day	14day	21 day
CT	_	335.7±7.22	325.5±4.06	305.4±6.31
HA	_	345.33±5.45	358.7±5.78	382.0±4.25
EAS	346.33±0.33	365.7±7.21	382.0±14.42	407.5±8.54
ACS	_	340.5±4.06	349.4±6.31	368.8±5.21
STD	348.7±7.44	380.7±15.9	396.0±3.06	445.0±6.21

Table:13..Ejaculation Latency CT, HA, EAS, ACS, STD

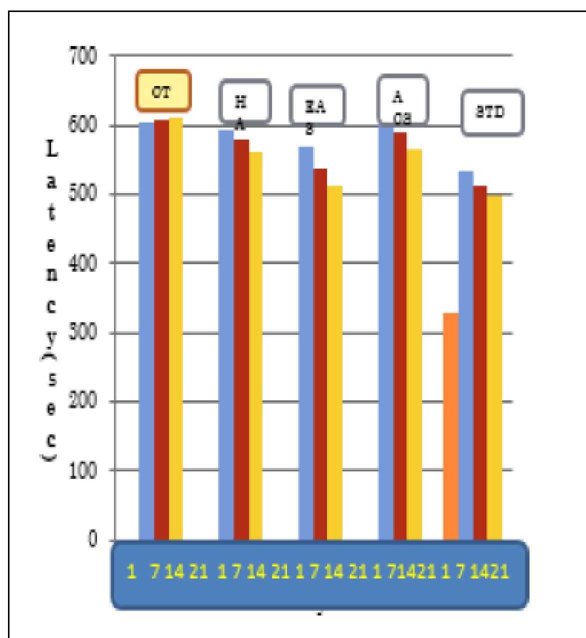


Graph:9.Ejaculation Frequency CT, HA, EAS, ACS, STD

Ejaculation Frequency

Group	0 day	7day	14day	21 day
CT	_	0.67±0.33	0.5±0.51	1.0±0.00
HA	_	0.67±0.33	1.33±0.67	1.67±0.67
EAS	0.33±0.33	1.00±0.58	1.67±0.33	2.00±0.33
ACS	_	0.67±0.33	1.33±0.67	1.0±0.54
STD	1.00±0.57	1.00±0.57	2.00±0.33	2.67±0.54

Table:14. Ejaculation Frequency CT, HA, EAS, ACS, STD



Graph:10. showing Post EjaculationLatency CT, HA, EAS, ACS, STD

Post ejaculation Latency

Group	0 day	7day	14day	21 day
CT	_	335.7±7.22	325.5±4.06	305.4±6.31
HA	_	345.33±5.45	358.7±5.78	382.0±4.25
EAS	346.33±0.33	365.7±7.21	382.0±14.42	407.5±8.54
ACS	_	340.5±4.06	349.4±6.31	368.8±5.21
STD	348.7±7.44	380.7±15.9	396.0±3.06	445.0±6.21

Table:15.Post Ejaculation Latency CT, HA, EAS, ACS, STD

XV.Result and Discussion

In ayurvedic system of medicine, *E.hirta* has long been in clinical use. The plant used in treatment of cough, asthma, diarrhea, piles, semen debility, worm infection, leprosy, skin diseases, urinary infection and disurea. It is aphrodisiac and enriches blood.etc. [3]

The present investigation deals with the study of pharmacognostic characters, extraction of dried leaves, preliminary phytochemical screening, quantitative estimation of phenolics and flavonoids, determination of antioxidant potential, TLC and HPTLC studies, pharmacological screening of crude extract and its fractions for aphrodisiac activity using mating behavior study.

Microscopic study was carried out to determined basic cellular composition of leaves and proves to be standards for identification of plant species. Various other physical properties like ash and extractive values were determined.

The dried leaves were defatted with petroleum ether and mark extracted with hydro alcohol. The crude extracts were concentrated in rotary vacuum dryer and the residue further fractionated with ethyl acetate and acetone fractions.

Preliminary phytochemical screening of extracts was carried out to reveal the presence of different primary and secondary metabolites. The hydro alcoholic extract revealed presence of steroids, tannins, flavonoids, saponins and carbohydrates. Ethyl acetate soluble fraction of hydro alcoholic extract showed presence of steroids, saponins, flavonoids, tannins and phenolics. Acetone soluble fraction showed presence of flavonoids, saponins, carbohydrates, tannins and phenolics. Acetone insoluble residue showed presence of carbohydrates and eliminated.

All extracts were subjected to quantitative estimation of total phenolics and flavonoids by Folin–Ciocalteu method and aluminum trichloride method respectively. The total phenolics content of HA, EAS and ACS was found to be 119.60,140.89 and 109.17 respectively and flavonoids content was found to be 104.57, 194.96 and 80.19 respectively.

All extracts were subjected to evaluation of antioxidant activity by DPPH free radical scavenging method. The conc. that inhibited 50% of free radicals were found 261.83, 95.05 and 318.40 for HA, EAS and ACS extracts respectively.

Mating behavior test was performed to evaluate the effect of the extracts on sexual behavior in active male rats.

The study exhibit a marked change in sexual behavior in male rats that were treated with standard drug or with 300 mg/kg of crude extracts of *E. hirta.* All the parameters of mating behavior test were observed on 0,7,14 and 21day.

Results revealed that ethyl acetate fraction of HA at dose 300 mg/kg. significantly increased the MF , IF and El . Also significant decreased in ML, IL, EF and PEI as compared to control and other extracts. However standard drug showed greater changes in these parameters.

With regard to the mechanism of action of the test drug, it is very difficult to explain the exact mechanism responsible for improving sexual functions. The drug induced changes in neurotransmitter level or their action at cellular level could change sexual behavior.

Ethyl acetate fraction and other extracts were found to have promising sexual behavior improving effect. So it was confirmed to be the active extract and subjected to TLC and HPTLC studies to estimate no. and type of phytoconstituents present in it. Numbers of solvent system were tried, however good resolution was obtained in the optimized solvent system. Presence of phytoconstituents was confirmed by spraying TLC plates with different spraying reagents.

XVI.Conclusion

Most of the natural plants in this review are those with aphrodisiac potentials. In this review some medicinal plants are used in Ayurveda formulations as aphrodisiac potentials to enhance performance as well as to increase vigor and vitality. Herbals drugs have a potential to treat the various types of body ailments. The demand of herbal drugs is increasing day by day in developed as well as developing countries because they are safer and well tolerated as compared to those of allopathic drugs. The information is recorded in plant's scientific name, common name of plant, family, part used for the aphrodisiac activity & reference. Scientists from divergent fields are investigating new plants with an eye to their aphrodisiac usefulness. These plants should be subjected to animal and human studies to determine their effectiveness.

In the present research work, attempts were made to study the probable antioxidant activity and aphrodisiac activity of different extracts of leaves of *Euphorbia hirta* Linn**.** . Work has clearly proved that the ethyl acetate fraction of hydro alcoholic extract have considerable antioxidant and aphrodisiac activity. Ethyl acetate fraction hydro alcoholic extract are found to contain tannin, flavonoids, sterols and saponins. The resultant significant and sustained increased in the sexual activity in male rats, suggests that *E.hirta* leaves possesses promising aphrodisiac activity, and also lend support to claims for its traditional usage as sexual function enhancing medicine. Thus it may prove to be an effective and safe alternative remedy in sexual disorder.

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