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RESEARCH ARTICLE

QUALITATIVE AND QUANTITATIVE ESTIMATION OF *EUPHORBIA THYMIFOLIA* LINN

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ABSTRACT

Euphorbia thymifolia linn (euphorbiaceae) it is an annual herb pan-tropic distribution. This is mainly found in waste land and along roadside wall sides under humid condition. Whole plant contains a crystalline alkaloidal principal allied to quercetin, 5,7,4-trihydroxy flavones 7-glucoside and essential oil. The plant is a rich source of phytochemicals like tannin, flavanoid, phytosterol, quercetin through lacking substantial phyto pharmacological studies. *Euphorbia thymifolia* total steroid EETS at dose 75 mg/kg showed a significant increase ($p \geq 0.001$) in sleeping time, depression of locomotor activity duration of tail suspension induced immobility ($p \leq 0.01$). Euphorbia thymifolia total phytosterol showed marked CNS depressant and sub-maximal, muscle relaxant activity and anxiolytic effect.

INTRODUCTION

Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available. (Gupta *et al*., 1998). According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times.

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originated from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine. (From the opium poppy) (Vickers *et al*., 1999). Medical history from the beginning of time is filled with descriptions of persons who used herbs to heal the sick of the society. However, parallel to the onset of the industrial revolution we witnessed the rise of allopathic medicine. Herbal medicine was also an effective healing method, but was viewed less enthusiastically.

Herbal products were discarded from conventional medical use in the mid-20th century, not necessarily because they were ineffective but because they were not as economically profitable as the newer synthetic drugs. In the early 19th century, scientific methods become more advanced and preferred, and the practice of botanical healing was dismissed as quackery. In the 1960s, with concerns over the iatrogenic effects of conventional medicine and desire for more self-reliance, interest in "natural health" and the use of herbal products increased. Recognition of the rising use of herbal medicines and other non-traditional remedies led to the establishment of the office of Alternative Medicine by the National Institute of Health USA, in 1992. Worldwide, herbal medicine received a boost when the WHO encouraged developing countries to use traditional plant medicine to filled needs unmet by modern systems. (Winslow *et al*., 1998)

Euphorbia thymifolia belong genus euphorbia which has many medicinal uses. The plant is bitter, acrid, sweet, thermo genic, laxative and diuretic. The plant is used from ancient times as antibacterial to treat ringworms, in snakebite, to treat dermatitis, eczema and skin inflammation. The leaves and seeds are given in bowel affection of children and are considered stimulant laxative. Euphorbia thymifolia linn have been reported to exhibit antiviral and antimicrobial activity. the plant is reported to contain quercetin, essential oil, tannin and different phyto sterols there is no substantial work done on the therapeutic potential of the plant so far therefore, the present study has been designed to explore the CNS activity profile of isolated phytosterol fraction.

MATERIALS AND METHODS

Collection of plant material

Herb was collected from, Bhopal (M.P.). The plant was collected in the month of September-October 2008. It was made completely clean, dust free and allowed to get dried under the shade.

Authentication of plant

Plant was identified and authenticated by Dr. Tariq Husain, Scientist and Head Herbarium and Angiosperm Taxonomy, National Botanical Research Institute. Lucknow (India) and a specimen voucher no. 97313 was assigned.

Drying and size reduction of plant material

The plant material was dried under shade. It was pulverised to coarse powder with the help of hand grinder. The coarse powder was packed into airtight container and stored in cool and dry place. This material was used for the further study.

Preparation of crude extract

250 gram of plant material was extracted with petroleum ether by hot maceration process for 24 hrs. The marc was pressed and filtered. The solvent was concentration under reduced pressure using rotator evaporator and dried below 4^0C. The extract was dark green in colour and having characteristic odour.

Extraction of plant by different solvents.

300 gram of crude *Euphorbia thymifolia* was extracted with different – solvent e.g. 90% ethanol, 70% hydro-alcohol, chloroform, benzene and hexane) by hot maceration. Filtered and dried, then salkowski and Lieberman burchard test was carried out on the extract- before and after hydrolysis with filter to explore the nature of non-polar component on steroid or triterpenoid.

This was designated as *Euphorbia thymifolia* total sterol (ETTS) and used further for in-vivo studies. Phytochemical test on ETTS showed positive result for the presence of steroid in Lieberman-burchard test and salkowski test and absence of saponins and alkaloid.

Separation of Phytosterol

Dried aerial part of plant
↓hot maceration
Extracted with pet. Ether
↓filter
Extracted lipid (filtrate)
↓volume reduction
Extracted lipid refluxed with 5% alcoholic KOH for 4 hr.
↓filter
Filtrate extracted with Di-ethyl ether
↓Di-ethyl ether layer
Dried and yield calculated
↓Positive test for steroid
Separated total phytosterol

Preparation of extract dosage form

Dosage form was made in 5% Tween 80. 1 gram of extract was taken in the motor and pestle and triturated with 10 ml of 5% of Tween 80 continuously to get homogenous suspension being concentration of 100 mg/ml with final drug concentration of tween 80, 50µl. Suspension was stored in airtight bottles in a cool place. Suspension of extract was administered by intraperitoneal route. All the standard drug solution was made in water for injection. (Kunanusorn *et al.*, 2009)

Animals

Male and female breed albino mice weighing between 25-35 gram and breed albino rat weighing between 150-200 gram are used in the experiments. The animals were placed randomly and allocated treatment group. All

the experiments were performed between 9:30 to 16:30 hours to overcome diurnal and circadian variations. All the animals were housed at a temperature of 24±2^{0}C and in a relative humidity of 65±5%. A 12:12 light day cycle was followed. All the animals were housed in polypropylene cages with paddy husk as bedding with free access to water and fed with standard commercial Pelleted chow (Hindustan Lever). All the experimental procedures and protocols used in this study were reviewed by institutional animal ethics committee of Radharaman College of Pharmacy proposal number IAEC/RCP/2017 and were in accordance with the guidelines of the IAEC.

Acute toxicity studies (LD_{50})

The acute toxicity studies were performed for *Lavandula stoechas* and *Dactylorhiza hatagirea* total extract using Swiss albino mice. The animals were fasted for 12 hour prior to the experiment and were administered intraperitoneal with different dose of extract dissolved in 5% Tween 80 (doses range from 50-1000 mg/kg^{-1} at various dose levels) and observed for mortality up to 48 h (Short term toxicity). Based on the short-term toxicity, the dose of next animal was determined as per OECD guideline 425. All the animals were also observed for long-term toxicity (14 days) (Diener *et al.*, 1995; OECD, 2000).

OBSERVATIONS

Tabale:-1 Petroleum ether extract was dissolved in $CHCL_3$ and tested for tannin and flavonoid.

S.no.	TEST	OBSERVATION	INFERENCE
01	**TANNINS**		
A	$Fecl_3$	No bluish black colored	**-ve**
B	Lead	No yellow ppt.	**-ve**
02	**FLAVONOIDS**		
A	Alkaline	No yellow colored formed	**-ve**
B	Lead acetate	No white ppt.	**-ve**
C	Zinc hydrochloride	No red colour	**-ve**

Tabale:-2 Qualitative analysis of *Euphorbia thymifolia* petroleum ether extract for presence of different phytoconstituents.

S.NO.	TEST	OBSERVATION	INFERENCE
01	**ALKALOIDS**		
A	Mayer's reagent	No cream colour ppt.	**-ve**
B	Dragendroff,s reagent	**No** reddish brown ppt.	**-ve**
C	Wagner,s reagent	No reddish brown ppt.	**-ve**
D	Hager,s reagent	No yellow ppt.	**-ve**
02	**GLYCOSIDE**		
A	General test	No red ppt. Was found in test A and B	**-ve**

03	**STEROID**		
A	Liebermann	Upper layer forms green and small reddish brown colour on lower layer	Steroid (++++)
B	Salkowski	Upper layer green	Steroid (++++)
04	**CARBOHYDRATE**		
A	Molisch test Before hydrolysis After hydrolysis	Upper layer green & lower layer dark reddish colour +lower layer dark green	**-ve**
B	Barfoed test Before hydrolysis After hydrolysis	Upper layer dark green + upper dark green and lower light green	
05	**TANNIS AND PHENOLICS**		
A	Ferric chloride test Before hydrolysis After hydrolysis	Upper layer green	Steroid (++++)
B	Gelatin test	No ppt. + Upper layer green and lower layer reddish brown	Steroid (++++)
06	**SAPONIN**		
A	Forth formation test	Lower layer hazy brown	**-ve**

Tabale:-3 Solubility Profile of EETS in different solvent

S.no.	Solvent	Solubility
1	Chloroform	++++
2	Di- ethyl ether	-
3	Acetic acid	-
4	Methanol	-
5	Alcohol	-
6	Acetone	-
7	Benzene	-
8	Glycerol	-
9	Tween 80	++++
10	PEG	+
11	Dimethyl sulfoxide	+
12	80% methanol water	-
13	Ethyl acetate	-

Tabale:-4 Phytochemical Sreening of separated EETS.

S.no.	TEST	OBSERVATION	INFERENCES
1	Liebermann burchard	Lower layer dark red colour	++++(steroid)
2	Salkowski	Lower layer dark red colour	++++(steroid)
3	Saponin	No dark red colour in lower layer	-ve
4	alkaloid	No dark red colour in lower layer	-ve

Tabale:-5 Test on different-solvent extract of *euphorbia thymifolia* to explore the nature of steroidal phytochemicals present.

Extract		70% hydro alcoholic	90% ethanolic	Benzene	Petroleum ether	Hexane
Before Hydrolysis		Light brick brown colour,(no layer separation)	Light brick brown colour with small ppt.	Dark green colour in lower layer	Dark bluish green ppt.	Upper layer light green (dark brown oily) sediment.
Liebermann Burchard	Observation					
	Inference	++	++	++++ (Steroid)	++++ (Steroid)	+++ (Steroid)
Salkowski	Observation	Light brown colour with light brown ppt.	Dark brick brown colour	Very dark green colour .(No layer separation)	Green colour in both layer (lower layer dark green colour)	Upper light yellowish green layer and dark brown oily sediment
	Inference	+++ (Steroid)	++++ (Steroid)	++++ (Steroid)	++++ (Steroid)	+++ (Steroid)
After Hydrolysis	Observation	Dark brick brownish colour on upper layer.	Dark brick brown colour, opaque	Dark bluish green colour,(no layer separation)	Dark brownish black ppt.	Upper layer light yellowish lower layer brown.
Liebermann Burchard	Inference	+++ (Steroid)	+++ (Steroid)	+++ (Steroid)	+++ (Steroid)	++++ (Steroid)
Salkowski	Observation	Very dark brown colour (no layer separation opaque)	Dark brown opaque colour.(no layer separation/opaque)	Dark blackish brown with bluish tint. (opaque, no layer separation)	Upper layer bright green,(lower layer golden yellow)	Upper layer light yellowish lower layer brown.
	Inference	+++ (Steroid)	+++ (Steroid)	++++ (Steroid)	++ (Steroid)	+ (Steroid)

Table:-6 Preparation of Calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (Mean) λmax=760 nm
0	0	0
1	25	0.740
2	50	0.855
3	75	0.954
4	100	1.050
5	125	1.151
Extract		0.069

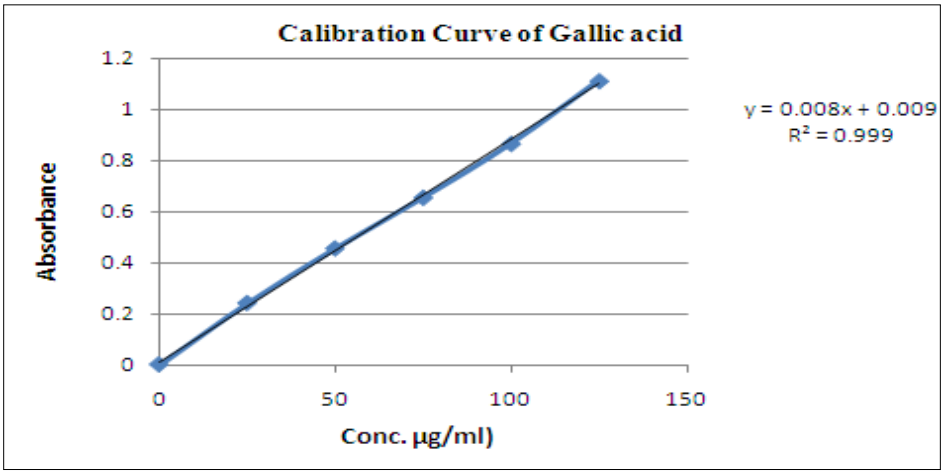

Figure:-1 Calibration curve of Gallic acid

Total flavonoid content estimation:

Tabale:-7 Preparation of calibration curve of Quercetin

S.no.	Concentration (µg/ml)	Absorbance (Mean) λmax=420 nm
0	0	0
1	25	0.234
2	50	0.448
3	75	0.658
4	100	0.869
5	125	1.102
Extract		1.328

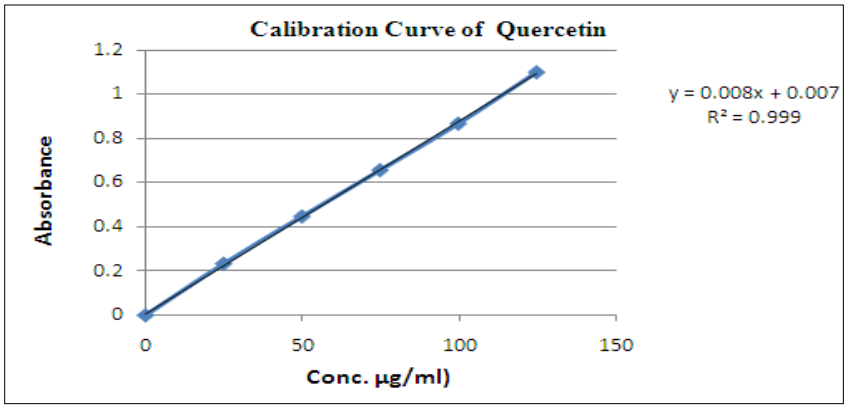

Figure:-2 Calibration curve of Quercetin

Tabale:-8 Quantitative estimation of phytoconstitutes

Phytoconstitutes	Values
Total polyphenol	0.676
Total flavonoid	4.201
Tannin	3.436

Tabale:-9 Ld_{50} determination of *Euphorbia thymifolia* steroidal fraction (log dose-probit) on mice.

Dose (mg/kg)	Log dose	% mortality	Correct% mortality	Probit value
1000	3	100	95	6.64
750	2.87	60	60	5.25
500	2.69	40	40	4.75
150	2.17	20	20	4.16
50	1.69	0	0	5.00

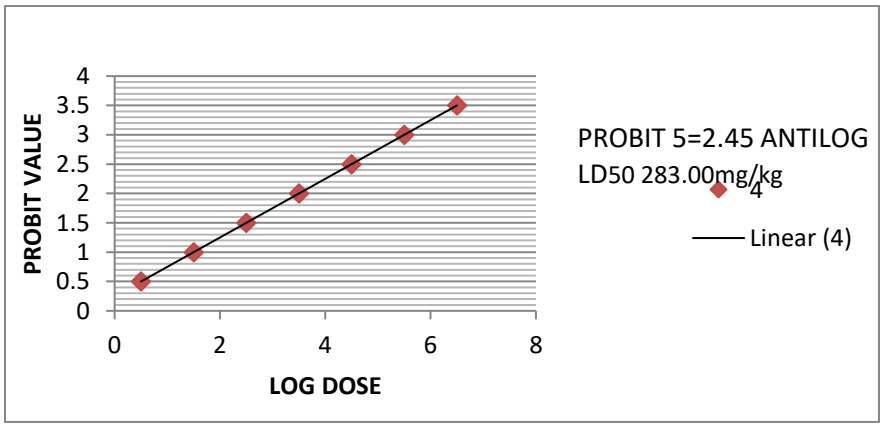

Figure 3- Graphical representation of log dose vs probit – value of *Euphorbia thymifolia* steroidal fraction.

EXPERIMENTAL RESULT

Qualitative analysis of *Euphorbia thymifolia* petroleum extract.

The percentage yield of petroleum ether extract was 2.5 % (6.4 gram). The petroleum ether extract of *Euphorbia thymifolia* shows the presence of steroid, tannins and phenolic compound. The alkaloid, glycoside, carbohydrate and saponins were absent. The results are shown in – 1 the petroleum ether extract was fractionated in $CHCL_3$ and tested for tannins, flavonoids. The tannin and flavonoids were absent in $CHCL_3$ fraction as shown in table:- 2 The different solvent extract of *Euphorbia thymifolia* like 70% hydro alcoholic, 90% ethanolic, $CHCL_3$, benzene, petroleum ether and hexane were tested for the presence of steroidal Phytoconstitutes. All the extract before hydrolysis and after hydrolysis showed in table:-3 the separated *Euphorbia thymifolia* total sterol was subjected to test for the presence of different Phytoconstitutes viz. Steroid, saponins and alkaloid. The EETS

gave positive result for only steroid. The result are shown in table:- 4. It confirms that the separated EETS has only steroid.

Quantative estimation of phytoconstitutes

The petroleum ether extract of *Euphorbia thymifolia* was explored for the quantitative estimation of total polyphenol, total flavonoid and tannins. The value of phytoconstituents were 0.676 mg/kg, 4.201 mg/kg and 3.436 mg/kg respectively for total polyphenol, total flavonoid and tannins as shown in table:-2

Acute toxicity study

Based on the OECD guidelines a limit test was performed to categorize the toxicity class (LD_{50}) of the compound. The limit test was performed at 500 mg/kg (I.P.), which showed mortality (40%). A main test was performed to determine the extract LD_{50} value following OECD up and down method. LD_{50} was calculated as 283.00 mg/kg from graphical presentation in figure. A dose range of 25, 50 and 75 mg/kg was selected for the evaluation of pharmacological activities as shown in table:-6

DISCUSSION

This study was conducted in the present Euphorbia thymifolia extract prepared in different solvents depending on polarity *Euphorbia thymifolia* was tested for the presence of different phytoconstituents. The plant was found to be rich in steroidal content so the extraction of total sterol was carried out. The steroids are the centrally active class of phytoconstituents which are responsible for many pharmacological activities.

Acute toxicity study was carried out on the total steroid extract of *Euphorbia thymifolia*. The estimated LD_{50} of Euphorbia thymifolia total steroid was 283 mg/kg indicating its moderately toxic properties. A dose range of 25, 50 and 75 mg/kg was selected for EETS to evaluated the pharmacological activity profile which ranges from $1/15^{th}$ to $1/5^{th}$ of LD_{50}.

Phytochemical analysis of *Euphorbia thymifolia* revealed the presence of alkaloid, saponins, flavonoids, triterpenes, tannins and steroids. *Euphorbia thymifolia* has rich presence of phytosterol with a yield of 10% (1.00gram). Antioxidant, antiviral, antifungal, diuretics, laxative, anti-diarrhoeal activities are reported in *Euphorbia thymifolia*.

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