Available online on 15.05.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Extraction, Phytochemical Screening, Isolation and Identification of Bioactive Compounds from Extract of the plant *Euphorbia Thymifolia* Linn

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ABSTRACT

Medicinal plants play an important role in the development of potent therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics as a source of many valuable secondary metabolites which serves as plant defense mechanisms against predator such as microorganism, insects and herbivores which have been proved to be potentially active compounds. *Euphorbia Thymifolia* Linn (*E. Thymifolia*) is commonly known as 'duddi' or in Sanskrit means Laghu didhika or Raktavindaka. It belongs to the family Euphorbiaceae. This plant is bitter, acrid, sweet and used as thermogenic, laxative and diuretic. This plant is widely used in the ayurveda to cure many diseases like vitiated condition of constipation, helminthiasis and ringworm skin diseases and leprosy. The aim of the present study is to examine *E. Thymifolia* Linn whole plant for phytochemical profile, **Isolation** and **Identification** of bioactive compounds. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. Isolation and characterization of bioactive compound from methanolic extract of *E. Thymifolia* has been conducted. The bioactive compound from methanolic extracts was isolated by several processes, such as TLC, column chromatography and preparative TLC. The isolated bioactive compound is identified by UV-Vis spectrophotometer, FT-IR, ¹H, ¹³C-NMR and Mass. The obtained compound is continued to the preparative TLC using chloroform: methanol (50:50, v/v) as eluent. The UV-Vis spectrum showed one peaks of maximum absorbance at 312.8nm. Then, the FT-IR spectrum showed several peaks that confirmed the presence of functional group of derivative of compound, i.e. 669.05, 928.58, 1070.85, 1215.51 and 1710.07cm⁻¹. ¹H and ¹³C-NMR spectrum confirmed the bioactive compound present in plant. Phytochemical analysis revealed the presence of alkaloids, glycosides, phenols, flavonoids, tannins. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Euphorbia Thymifolia*, Qualitative phytochemical, Isolation, Bioactive compounds

Article Info: Received 18 March 2019; Review Completed 2 April 2019; Accepted 24 April 2019; Available online 15 May 2019

Cite this article as:

Shrivastava R, Mishra J, Extraction, Phytochemical Screening, Isolation and Identification of Bioactive Compounds from Extract of the plant *Euphorbia Thymifolia* Linn, Journal of Drug Delivery and Therapeutics. 2019; 9(3):107-113
<http://dx.doi.org/10.22270/jddt.v9i3.2608>

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INTRODUCTION

Ayurveda stresses on the use of vegetable drugs. Plants are being used as medicine since ancient Times^{1,2}. A numbers of bioactive compounds in medicinal plants, such as alkaloids, tannins, flavonoids, sterols, triterpenes, etc., are noted to play major role in physiology and management of diseases³. Flavonoids constitute one of the most exclusive classes of compounds in medicinal plants⁴. The foremost important task in this paradigm is the screening of flavonoids in plants. Phenolic compound are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom, even if the type of compound present varies according to the phylum under consideration. Phenolic are uncommon in varies bacteris, fungi and algae. Bryophytes are regular producers of polyphenols including flavonoids, but it is in the vascular plants that the full range of polyphenol is found⁵. Phenolic substances or polyphenols contain numerous varieties of compounds: simple

flavonoids, phenolic acids, complex flavonoid and colored anthocyanins. These phenolic compounds are usually related to defense responses in the plant. However, phenolic metabolites play an important part in other processes, for instance incorporating attractive substance to accelerate pollination, coloring for camouflage and defense against herbivores, as well as antibacterial and antifungal activities⁶. Chromatographic studies of these compounds serves to be a very useful and reliable source in the process of bioactive compounds screening in plants⁷. According to the ethnobotanical information, it has been reported that the plant *E. Thymifolia* (family- Euphorbiaceae) is a medicinal herb used traditionally in dysentery, bleeding piles, gonorrhoea, dysmenorrhoea, amenorrhoea, helminthiasis, ringworm, chronic cough, asthma, bronchitis, cardiac debility, greying of hairs, skin diseases etc^{8, 9}. *E. Thymifolia* have numerous pharmacological activities including antibacterial^{10, 11}, antifungal¹², antimicrobial¹³, anti-inflammatory^{14,15}, antiviral¹⁶, antispasmodic, bronchodilator,

antibronchial, asthmatic¹⁷⁻¹⁹, hypoglycaemic²⁰⁻²², anticancer^{23,24} and antioxidant²⁵. A number of chemical constituents are present in *E. Thymifolia* whole herb and its different parts. According to the literature, the whole plant contains epitaraxerol, n-hexacosanol, euphorbol, 24-methylene cycloartenol, 12-deoxy-4P-hydroxyphorbol-13-dodecanoate-20-acetate, 12-deoxy-4P-hydroxyphorbol-13-phenylacetate-20-acetate, 12-deoxyphorbol-13,20-diacetate, quercetin-3P-galactoside, 12-deoxyphorbol-13,20-diacetate, 12-deoxy-4Phydroxyphorbol-13-dodecanoate-20-acetate, n-hexacosanol, esters, n-alkanes and sterols^{26,27}. Roots contain taraxerol and triucalol. Leaves and stems consist of 4-trihydroxy flavone-7- glycoside²⁶. Leaves contain ellagitannin dimers, euphorbins G and H with 12 known polyphenols²⁷⁻³². Therefore, the aim of this research is extraction, isolation, phytochemical screening and identification of bioactive compounds from methanolic extract of *E. Thymifolia* whole herb through several processes, such as thin layer chromatography, column chromatography and preparative TLC. To identify the structure of bioactive compound, several analyses has been conducted such as UV-Vis, FT-IR, ¹H, ¹³C-NMR and mass.

MATERIAL AND METHOD

Plant material

The whole plant material of *E. Thymifolia* was collected from the Betwa riverine zone of Vidisha during rainy seasons of the year 2014 and washed thoroughly with distilled water. It was identified and authenticated by Taxonomist Dr. Sunil Dubey, Department of Botany, St. Mary P. G. College, Vidisha (M.P.). A voucher specimen was procured which was deposited in Department of Botany and Microbiology, St. Mary's P. G. College, Vidisha (M. P.) for future reference and the specimen voucher no. of *E. Thymifolia* is 104105.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction Procedure

Defatting of plant material

Plant material of *E. Thymifolia* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with n-Hexane using soxhlation method. The extraction was continued till the defatting of the material had taken place.

Extraction

100gm of dried plant material were exhaustively extracted with different solvent in increasing order of polarity i.e. n-Hexane, petroleum ether, benzene, acetone, methanol and distilled water using Soxhlet apparatus for 4 days. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts. Dried extract was collected in an air tight container and stored at 4°C for further analysis³³⁻³⁶.

Qualitative analysis of phytochemicals

The methanolic extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence or absence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids in

methanolic extracts of *E. Thymifolia*. The above phytoconstituents were tested as per the standard method³⁷.

Fractionation of the extract by thin layer and column chromatography

Thin layer chromatography

The presence of different phytoconstituents in the methanolic extract of plant *E. Thymifolia* was established by TLC on silica coated alumina plates G' 60 F254 plate of 0.2 mm thickness by using different solvent systems as mobile phase. The selective solvent system used for the phytoconstituents detection was Chloroform (50): Methanol (50). A spotting capillary was used to add the extract to the plate. To spot the plate, simply touched the end of the capillary tube to the coated side of the plate and placed the TLC plate in the development chamber. The bottle was filled with a small amount of the mobile phase of solvent system and capped with a cork till complete development. After the complete development of spots on TLC plates, UV Chamber under the wavelength 254 nm of UV light was used to visualize the spots and the spots color were noticed. Besides this, Iodine chamber and visible light was also used to visualize the spots. RF (retention factor) values of the fractions were measured by applying formula of Brimley and Barrett^{38as}:

$$R_f = \frac{\text{Distance travelled by the solvent}}{\text{Distance travelled by the solute}}$$

Column chromatography

In the present study, silica gel was used as a stationary phase which was poured in to the column then filled chloroform solvent as mobile phase and was run two three times for maintaining its flow by removing air bubbles entered during packaging of silica. Then loaded sample on the top of the column and filled the column with appropriate solvent systems. The selective solvent system used for phytoconstituents detection was Chloroform: Methanol (50:50). Then, obtained purified fractions were separated on basis of their color characterization and were kept in separate vials for further analysis.

Isolation and structural elucidation of the compound

The biologically active purified fractions of the extract were sent to PBRL (Pinnacle Biomedical Research Laboratory), Bhopal for spectral analysis (UV) and to the SAIF CDRI, Lucknow for spectral analysis (IR, HNMR, CNMR and Mass) and spectral graphs were obtained. Which were interpreted with the literature available in books of spectroscopic analysis^{39,40}.

RESULTS AND DISCUSSION

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction.

Table 1: % Yield of plant material

S. No.	Solvents	<i>Euphorbia Thymifolia</i>
1.	N- hexane	3.65%
2.	Pet. Ether	1.56%
3.	Benzene	2.96%
4.	Methanol	7.34%
5.	Aqueous	4.38%
6.	Acetone	3.83%

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1.

A small portion of the dried extracts were subjected to the phytochemical test using standard methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. The outcomes of the results are discussed separately in the table 2.

Table 2: Preliminary phytochemical screening of *Euphorbia Thymifolia* (Linn.) extracts.

S. No.	Tests	Phytochemicals	Results
1	<ul style="list-style-type: none"> • Mayer's test • Wagner's test • Dragendorff's test • Hager's test 	Alkaloids	+
2	<ul style="list-style-type: none"> • Molisch's test • Benedict's test • Fehling's test 	Carbohydrates	+
3	<ul style="list-style-type: none"> • Modified Borntrager's test • Legal's test 	Glycosides	+
4	• Froth/Foam test	Saponins	-
5	<ul style="list-style-type: none"> • Salkowski's test • Libermann-Burchard test 	Steroids	+
6	Gelatin test	Tannins	+
7	<ul style="list-style-type: none"> Alkaline Reagent test • Lead acetate test 	Flavonoids	+
8	<ul style="list-style-type: none"> • Xanthoproteic test • Ninhydrin test 	Proteins and amino acids	-
9	Test for Triterpenes	Triterpenes	+

+ (present), - (Absent)

In the present study, thin layer chromatography of *E. Thymifolia* whole plant crude methanolic extract was done and three spots of solutes were seen on the plate between the distance travelled by the solvent system (7.3 cm). These

fractions of solutes were observed at 2.5cm, 5cm and 6.3cm distances, respectively. Finally, RF (retention factor) values of the fractions were measured and given in table 3.

Table 3 Thin layer chromatography of *Euphorbia Thymifolia* plant extract

Plant Extract Of <i>Euphorbia thymifolia</i>	Solvent System Used	No. of spots	RF Value	Color characterization		
				Visual light	UV-light	Iodine chamber
Methanol	Chloroform (50) : Methanol (50)	ET-1	0.34	Light yellow	Bluish yellow	Dark Yellow
		ET-2	0.68	Yellow	Dark yellow	Dark brown
		ET-3	0.86	Brownish green	Bluish green	Brownish green

After the confirmation of solvent system applied in thin layer chromatography and obtained fractions of the extracts, the huge amount of extract was uploaded in column with the same solvent system after running column of silica gel with chloroform: methanol (50:50) solvent to separate the huge

amount of the fractions of the extracts to use it for experimental bioassay. Four fractions of dark green, light green, pale yellow and red were obtained which were further used for experimental bio-assay and spectral analysis was given in table 4.

Table 4 Column chromatography of *Euphorbia Thymifolia* plant extract

Whole plant extract	Solvent system used	Fractions obtained	Color characterization
<i>Euphorbia thymifolia</i>	Chloroform:Methanol (50:50)	ET-1 ET-2 ET-3 ET-4	Dark green Light green Pale Yellow Red

In the present study, biologically active purified fraction (ET-2) of the *E. Thymifolia* whole plants methanolic extracts was analysed for the detection and confirmation of the bioactive compound present in the fraction. Sample of the purified fraction (ET-2) of *E. Thymifolia* was measured by using

systronics double beam UV-VISIBLE Spectrophotometer Model 2202. Peaks as shown in fig. 1. Maximum absorbance of the extract 1.71 was reported at $\lambda_{\max}$ 312.8nm and minimum absorbance 0.511 was reported at $\lambda_{\max}$ 274.4nm wavelength (in 95% ETOH). The structured secondary band

which appeared as broad band at 312.8nm along with 1.71 absorbance confirms the presence of cyclohexane like compound. Another broad band appeared at $\lambda_{\max}$ 274.4nm

along with 0.511 absorbance confirms the presence of cyclopentane like compound in the purified fractions.

Figure 1: UV spectra of purified fraction (ET-2).

Sample ET-2 of *E. Thymifolia* was uploaded in Perkin Elmer Spectrum (Version 10.03.06) at SAIF, CDRI, Lucknow, (UP) by administrator to get IR spectra of the compound. Total 13 peaks were reported in the sample (ET-2) of *E. Thymifolia* MEOH fraction as shown in fig 2 which described that in finger print region ($<1500\text{cm}^{-1}$) 7 peaks were observed. The smallest peak at 669.05cm^{-1} belongs to the stretching vibration due to the presence of O-H group. Another peak at 757.75cm^{-1} confirmed the presence of aromatic ring compound isomer especially on its ortho position in aromatic ring ($770\text{--}735\text{cm}^{-1}$). The peak at 928.58cm^{-1} showed Carbon Hydrogen banding vibration. The peak at 1070.85cm^{-1} explains the strong intensity of C-O stretching. The peak at 1215.51cm^{-1} reports medium intensity of -C-N.

A peak at 1384.12cm^{-1} explains carbon hydrogen banding vibration CH_3 . One more peak at 1640.96cm^{-1} indicates for Aromatic ring compound. Besides this, a peak at 1710.07cm^{-1} explains strong intensity of double bond between Carbon and Oxygen (C=O). Moreover, a peak at 2399.74cm^{-1} describes strong and very broad intensity of carboxylic acid. A peak at 2855.13cm^{-1} also confirms the presence of cyclopentane and hexane like compound that is probably λ -Lactone. A small blunt peak at 2927.9cm^{-1} shows strong Carbon-Hydrogen stretching vibration (C-C-H), a peak at 3019.12cm^{-1} depicts O-H group stretching and finally a last peak at 3408.99cm^{-1} describes strong and broad intensity of OH-group.

Figure 2: FT-IR Spectrum of purified fraction (ET-2)

The ^1H NMR (Agilent, CDCl_3 , 300MHz) peaks observed at different concentration (ppm) describes the position of hydrogen in the compounds. Three small peaks were reported between 80.754 to 0.787 ranges signify for 3H (s) and eight medium peaks were reported between 80.803 to 80.898 ranges signify for 3H (s). Besides this, five peaks of small size were seen between 80.920 to 80.976 (2H, m), and twenty seven peaks between 81.001 to 81.730 were reported out of these, 81.29 (16H, m), 81.30 (2H, s), 81.50 (3H, s), 81.62 (2H, m). Moreover, eight peaks were reported between the ranges of 82.020 to 82.806. Out of these peaks, 82.03 (2H, m), 82.05 (2H, m), two peaks were seen of 85.352 (1H, m), 85.362 (1H, m) and finally, one peak of 87.265 was seen indicates for (2H, d, $J=0.75\text{Hz}$) as shown in fig 3 which confirms the presence of sesquiterpene lactones in the

purified fraction. The ^{13}C NMR (Agilent, 300MHz) peaks observed in the fig 4 at various concentration depicted the position of carbon and hybridization in the compound. First peak was of $\delta 12.17$ (q, sp^3 , CO- CH_3), two peaks of $\delta 14.32$ and $\delta 14.47$ (t, sp^2 -sp, O- CH_3), two peaks of $\delta 16.16$ and $\delta 16.30$ (d, sp, O- CH_3), two peaks of $\delta 18.19$ and $\delta 18.50$ (t, C-3, sp^2 , 2H, CH_2), three peaks of $\delta 19.23$, 19.51 and 19.94 (t, sp^2 , C-3, 2H, CH_2), two peaks of $\delta 20.01$ and $\delta 20.74$ (t, sp^2 , C-10, 2H, CH_2), one peak of $\delta 21.27$ (t, sp^2 , C-3, C-5, 2H, CH_2), three peaks of $\delta 22.18$, $\delta 22.28$ and $\delta 22.89$ (t, sp^2 , C-1), two peaks of $\delta 24.66$ and $\delta 24.95$ and five peaks of $\delta 25.22$, $\delta 25.62$, $\delta 25.72$, $\delta 25.81$ and $\delta 25.93$ (d, sp, CH), one peak of $\delta 26.25\text{ppm}$, two peaks of $\delta 27.39$ and $\delta 27.79$ (s, C), two peaks of $\delta 28.17$ (t) and $\delta 28.44$ (t), six peaks of $\delta 29.30$ (t), $\delta 29.36$ (t), $\delta 29.47$ (t), $\delta 29.56$ (t), $\delta 29.66$ (t) and $\delta 29.90$ (t), one peak of $\delta 30.98$ (t),

one peak of $\delta 31.72$ (t), two peaks of $\delta 32.12$ (t) and $\delta 32.93$ (t), one peak of $\delta 34.15$ (t), one peak of $\delta 36.34$ (t), two peaks of $\delta 37.48$ (t) and $\delta 37.63$ (t), one peak of $\delta 39.56$, one peak of $\delta 43.01$, one peak of $\delta 62.30$ (q), one peak of $\delta 63.23$ (q), one

peak of $\delta 76.81$ (s), three peaks of $\delta 77.23$ (s), $\delta 77.43$ (s) and $\delta 77.65$ (s), two peaks of $\delta 127.30$ (d) and $\delta 127.94$ (d), one peak of $\delta 128.48$ (d), one peak of $\delta 130.41$ (d), one peak of $\delta 132.14$ (s) and last peak of $\delta 178.69$ (s).

Figure 3: ^1H NMR spectra of purified fraction (ET-2)

Figure 4: ^{13}C NMR spectra of purified fraction (ET-2)

Mass spectrum of purified fraction of *E. Thymifolia* (ET-2) was analyzed for detection of biologically active compound on mass spectrometer and peaks were observed in the fig 5. In ESIMS, first peak was reported at 118.0860 (m/z) that confirms the presence of Phenyl Propene or Allyl Benzene or Indane with molecular formula C_9H_{10} (**Fig. 6A**). A prominent spectral peak at 149.0232m/z confirms the presence of 2-hydroxy pentane-dioic acid with molecular formula $C_5H_8O_5$ (**Fig.6B**). A largest peak reported at 391.2853m/z depicts the presence of Di-N-Octyl phthalate with molecular formula $C_{24}H_{38}O_4$, (**Fig.6C**). A large peak at 279.1578m/z confirms the presence of 2-methoxyisoxazolidine-3,3, 5, 5-tetra

carboxylic acid with molecular formula $C_8H_9NO_{10}$, (**Fig.6D**). A large peak reported at 441.3737m/z confirms the presence of 4 α -Carboxy-4 β -Methyl-5 α -Cholesta-8, 24-dien-3 β -ol with molecular formula $C_{29}H_{45}O_3$. (**Fig.6E**). One of the peak reported at 593.2726m/z confirms the presence of 2-(5'-O-Methyl-3'-YL)-N-[(S)-1-(2'- α -Methoxy-3'- α -Methyl-5'-Deoxythymidine-5'-YL)Ethyl] Acetamide with molecular formula $C_{27}H_{39}N_5O_{10}$. (**Fig.6F**). Rest of the peaks was also seen in the mass spectrum which was found due to fragmentation of terpenoidal compound present in the bio-active compound.

Figure 5: MASS Spectra of purified fraction (ET-2)

Figure 6: (A) Phenyl Propene (B) 2-Hydroxypentanedioic acid (C) Di-N-Octylphthalat (D) 2-methoxyisoxazolidine-3,3,5,5-tetracarboxylic (E) 4alpha-Carboxy-4beta-methyl-5alpha-cholesta-8,24-dien-3beta-ol (F) 2-(5'-O-Methyl-3'-Deoxythymidin-3'-yl)-N-[(S)-1(2'Alpha-Methoxy-3'-O-Methyl-5'-Deoxythymidin-5'-yl) Ethyl]Acetamide

CONCLUSION

From the result obtained it can be concluded that the whole plant of *E. Thymifolia* has medicinal values since it contains more secondary metabolites. Bioactive compound (Total 6) were isolated and identified by column, thin layer chromatography and which were subjected to spectral analysis i.e. IR, UV, 1D NMR (1H-NMR and 13C-NMR) and mass spectroscopy from methanol extract of *E. Thymifolia* (ET-2) whole herb. These compounds have been reported for the first time in this plant and can serve as a useful tool in its standardization. Methanolic extracts shows good results regarding presence of phytoconstituents hence these plants may directly use in medicine preparation or for the development of novel agents for various pathological disorders. Further research on the health benefits of phytochemicals in this plant may be warranted.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Ajithabai MD, Sunitha Rani SP, Jayakumar G. Review on the species of *Sida* used for the preparation of *nayopayam kashayam*. *Int J Res Rev Pharm Appl Sci* 2012; 2:173-95.
- Visht S, Chaturvedi S. Isolation of Natural Products. *Curr Pharm Res* 2012; 2(3):584-599.
- Mishra GJ, Reddy MN, Rana JS. Isolation of Flavonoid Constituent from *Launaea procumbens* Roxb by Preparative HPTLC Method. *IOSR J Pharma* 2012; 2(4):5-11.
- Samuelson G. *Drugs of Natural Origin*, A textbook of Pharmacognosy. 5th ed. Stockholm: Swedish Pharmaceutical Press. 2004.
- Latanzio V. *Phenolic Compound: Introduction* Springer-Verlag Berlin Heidelberg 2013; 1543-1579.
- Derong L, Xiao M, Zhao J, Li Z, Xing B, et al. Review An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules* 2016; 21: 1374.
- Gadhvi R, Reddy MN, Gaurav JM. Isolation of terpenoids constituents from *Lippia nodiflora* by preparative HPTLC method. *Int J Med Plants Alter Med* 2013; 1(3):104-9.
- Mitra R. *Bibliography on Pharmacognosy of Medicinal Plants*, National Botanical Research Institute, Lucknow, 1985; 239.
- Siddiqui MB, Husain W. Traditional treatment of gonorrhoea through herbal drugs in the province of Central Uttar Pradesh, India. *Fitoterapia* 1993; 64(5):399-403.
- Khan NH, Rahman M, Nur-e-Kamal MSA. Antibacterial activity of *Euphorbia thymifolia* Linn. *Indian J Med Res* 1988;87(6):395-7.
- Banerjee DK, Pal DC. Plants used by the tribal of plain land in India for hair and scalp preparation, Fourth Int. Congr. Ethnobiol., NBRI, Lucknow 1994; 17(21):340.
- Pal S, Gupta I. In vitro studies on the antifungal activity of *Chotidudhi* plant (*Euphorbia prostrata* Ait. and *Euphorbia thymifolia* Linn.). *Indian J Pharmacol* 1971; 3:29.
- Jabbber A, Khan GMAS. Antimicrobial alkaloids from *Euphorbia thymifolia*. *Pak J Sci Ind Res* 1965; 8(1):293-4.
- Singla AK, Pathak K. Anti-inflammatory studies on *Euphorbia pmstrata*. *J Ethnopharmacol*. 1989; 27(1):55-61.
- Singla AK, Pathak K. Topical anti-inflammatory effects of *Euphorbia prostrata* on carrageenan induced foot pad oedema in mice. *J Ethnopharmacol* 1990; 29(3):291-4.
- Manickam K, Rajappan K. Inhibition of antiviral activity of certain leaf extracts against tomato spotted wilt virus in cowpea. *Ann Plant Prot Sci* 1988; 6(2):127-30.
- Sharma GD, Tripathi SN, Sharma CD. Evaluation of a herbal drug "Dugdhika" (*Euphorbia prostrata* W. Ait. and *E. thyrnifolia* Linn.) for the treatment of bronchial asthma (Tarnaka shvasa). *Asian Conf Trad Asian Med*, Bombay, India. 1983; Abstr. No. C-60.
- Sharma GD, Tripathi SN. Experimental evaluation of Dugdhika (*Euphorbia prostrata* W. Ait) for the treatment of 'Tarnaka Svasa' (Bronchial asthma). *Ancient Sci Life* 1983; 3(3):143-50.
- Sharma GD, Upadhyay BN, Tripathi SN. A clinical trial of *Euphorbia prostrata* W. Ait and *E. thyrnifolia* Linn. in the treatment of bronchial asthma (Tarnaka shvasa). *J Res Ayur Siddha* 1982; 3(3-4):109-18.
- Akhtar MS, Khan QM, Khaliq T. Effects of *Euphorbia prostrata* and *Fumaria parviflora* in normoglycaemic and alloxan-treated hyperglycaemic rabbits. *Planta Medica* 1984; 50(2):138-42.
- Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as anti-diabetics. *J Ethnopharmacol* 1998; 61(2):101-10.
- Handa SS, Chawla AS, Maninder. Hypoglycaemic plants-A review. *Fitoterapia* 1989; 60(3):195-224.

23. Baslas RK, Agarwal R. Chemical investigation of some anticancer plants of Euphorbia genus. Indian J Chem 1980; 19B(8):717-8.
24. Sabu P, Saheed S, Ghouse AKM. Air pollution hazard on an indigenous drug plant Euphorbia thymifolia Linn. Medicinal Plants: New Vistas of Res. 1993; 11(2):549- 53.
25. Lin CC, Cheng HY, Yang CM, Lin TC. Antioxidant and antiviral activities of Euphorbia thymifolia L. J Biomed Sci 2002; 9(6):656-64.
26. Makoto N. Flavonol glucoside from Euphorbia thymifolia L. J Agr Chem Soc Japan. 1941; 17:483-484. Chem Abstr, 1942, 3625.
27. Gupta DR, Garg SK. Chemical examination of Euphorbia thymifolia. Indian J Appl Chem 1966; 29(1):39-40.
28. Behari M, Gupta M. Chemical analysis of Euphorbia thymifolia Linn., Proc. 67th Session, Indian Sci. Congr., Calcutta, Part I11 (Chemistry Section), Abstr. No. 214, 1980.
29. Agarwal R, Baslas RK. Chemical examination of the aerial parts of Euphorbia thymifolia. Indian J Pharm Sci 1981; 43(9):182-3.
30. Lee SH, Tanaka T, Nonaka G, Nishioka I. Hydrolysable tannins from Euphorbia thymifolia. Phytochemistry 1990; 29(11):3621-5.
31. Singla AK, Pathak K. Phytoconstituents of Euphorbia species. Fitoterapia 1990; 61(6):483-516.
32. Asolkar LV, Kakkar KK, Chakre OJ. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles, Publications and Information Directorate, CSIR, New Delhi, Part I (A-K). 1992; 307.
33. Kokate CK Practical Pharmacognosy, 3rd Edition, Vallabh Prakashan, New Delhi, 1991; 101-111.
34. Harborne JB. Phytochemical Methods, a guide to modern techniques of plant analysis, Chapman & Hall, London, 1973; 3:182-189.
35. Trease GE, Evans WC. Pharmacognosy. 12th Edition, ELBS Publications, London, 1985, 126-137.
36. Brady LR, Robber JE. Pharmacognosy 9th edition, Lea and Febiger Publication, Philadelphia, 1988: 77-79.
37. Peach K, Tracy MV. Modern Methods of Plant analysis, Vol.3&4, Springer-Verlag, Heidelberg. 1955; 3-4.
38. Brimley RC, Barrette FC. Practical Chromatography. Chapman and Hall Publication, London. 1953; 26.
39. Rao CNR. Ultraviolet and Visible spectroscopy. 1975
40. Silverstein RM, Webster FX, Kiemle DJ. Spectrometric Identification of organic compounds. 6th Edition. 1997.