International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
October - November, 2013, Vol. 2, No.6, pp 699-704
ISSN: 2278-0238

Research Article

QUANTITATIVE ESTIMATION OF LUPEOL IN POLYHERBAL FORMULATION AND SUCCESSIVE EXTRACTS OF *CRATAEVA NURVALA* AND THEIR *IN VITRO* ANTI-INFLAMMATORY ACTIVITY STUDY

R. Jayaprakasam *[1], S. Velayutham[1], B. Asha jyothi[1], Namitha philips[1], T. K. Ravi[1]

1. College of Pharmacy, Sri Ramakrishna Institute Of Paramedical Sciences, Coimbatore -641 044.

***Corresponding Author: Email** jprcognosy@gmail.com

(Received: August 02, 2013; **Accepted:** September 21, 2013**)**

ABSTRACT

The aim of the present study is to carry out the quantitative estimation of lupeol in poly herbal formulations by HPTLC and to study the *invitro* anti-inflammatory activity study of *Crataeva nurvala* bark. The bark of *Crataeva nurvala* was selected, dried, powdered and extracted with petroleum ether, diethyl amine and methanol successively by continuous hot percolation method using soxhlet apparatus. Preliminary phytochemical screening of the successive extracts showed the presence of chemical constituents like triterpenoids, flavonoids, alkaloids, tannins, glycosides and sesquiterpenes. The R_f value of lupeol was found to be 0.49 ± 0.03.The developed methods were validated as per ICH guidelines. The LOD and LOQ was found to be 50 and 300ng/spot respectively for HPTLC. The anti-inflammatory activity was highest in petroleum ether extract when compared to other extracts when evaluated by using ibuprofen as the control. This shows that the polyherbal formulation and medicinal plants containing lupeol as the active constituent has medicinal value and that they may be utillised for their anti-inflammatory properties.

Keywords: *Crataeva nurvala*, lupeol, HPTLC, ascorbic acid.

INTRODUCTION

Traditional systems of medicine have been steadily gaining interest and acceptance all over the world, even among the practitioners of modern medicine. Consequently plant materials and herbal drugs derived from them represent a substantial proportion of the current global drug market. In this scenario, there is a need to ensure the quality of herbal preparations. The only way to ensure that herbal drugs and preparations made with them achieve optimum and consistent quality is to create and maintain a comprehensive quality assurance system[1]. The plant *Crataeva nurvala* belonging to Capparidaceae family and its bark were slightly bitter. The bark of *Crataeva nurvala* was selected for the analytical work because *Crataeva nurvala* bark contains high amounts of triterpenoids, sesquiterpene, alkaloids, tannins which are

responsible for the main pharmacological actions like anti-oxidant, anti-inflammatory activity, etc[2]. It is used in genitourinary conditions such as benign prostatic hyperplasia, chronic urinary tract infections, atonic bladder, prevention of kidney stones, atonic bladder, hypotonic bladder and as a bladder tonic and for prevention and treatment of kidney stones[3]. The solvents of increasing polarity selected were petroleum ether, diethyl amine and methanol. The extracts were subjected to preliminary phytochemical screening, HPTLC quantification for the marker and *invitro* ant-inflammatory activity studies. In this study, the formulation

which was selected(HIMPLASA TABLETS) contained 80mg of *Crataeva nurvala* for quantitative estimation of lupeol by validated HPTLC.

Materials and methods

Plant material

The collected whole Plant was identified and certified by Dr.C.Kunhikannan, Scientist D, IFGTB, Coimbatore, Tamil Nadu. The authentified certificate of the plant *Crataeva nurvala* is enclosed.

Process of extraction

The powder was extracted with various organic solvents viz., petroleum ether, diethyl amine and methanol successively by continuous hot percolation method using soxhlet apparatus. The duration of each extraction was 5 days to get a well extracted product by the respective solvents. After each extraction, the extract was collected & dried either under air at room temperature or by by heating the extract at temperature below the boiling point of the extracting solvent to get a well dried extract. Then the dried extract was weighed and the percentage yield of the extract from the weighed bark powder was calculated. The extracts were stored in a refrigerator at 4^{o}C until further analysis. The collected extracts were subjected to preliminary phytochemical screening, TLC, & HPTLC analysis and *in vitro* anti-inflammatory activity studies.

High Performance Thin Layer Chromatography

Preparation of standard solution

Stock solution of lupeol (50µg/mL), was prepared in methanol.

Preparation of sample solution for polyherbal formulation

Ten tablets, each containing 80 mg of *Crataeva nurvala* were weighed and transferred to round bottom flask, 100 ml of methanol was added to the mixture and tablets was refluxed for one hour. The tablets were extracted with further 50ml quantities of methanol till the extract becomes colourless. The extracts were pooled and evaporated under vaccum on rotary evaporator, to form a thick paste which was mixed with 40ml of hot water. The aqueous part of the extract was taken and it was extracted continuously with 50ml of ethyl acetate. The ethyl acetate extract was concentrated on a rotary evaporator under vaccum to dryness. The final residue obtained was dissolved in 50ml methanol and evaporated to dryness under vacuum.

Preparation of sample solution for petroleum ether extract

10 mg of petroleum ether extract was weighed and dissolved in 10 ml of petroleum ether and it was filtered through whatman filter paper to remove the solid particles.

Preparation of sample solution for diethyl amine extract

10 mg of diethyl amine extract was weighed and dissolved in 10 ml of diethyl amine and it was filtered through whatman filter paper to remove the solid particles.

Preparation of sample solution for methanol extract

10 mg of methanol extract was weighed and dissolved in 10 ml of methanol and it was filtered by whatman filter paper to remove the solid particles.

Fixed chromatographic parameters

Stationary Phase : Pre-coated silica gel 60F254 on aluminium sheets.

Mobile phase : toluene : methanol (9 :1,%v/v)

Chamber saturation time : 20 minutes

Migration distance : 80 mm

Detection wavelength : 366 nm

Derivatizing agent : Anisaldehyde sulphuric acid

VALIDATION OF HPTLC METHOD

Linearity and Range

Linear regression data revealed an excellent linear relationship in the concentration range of 300 to 900ng/spot. The slope, intercept and correlation co-efficient values were found to be 190.577, 1.110 and 0.99845.

Accuracy

Recovery studies were done for determining accuracy parameter. It was done by mixing known quantity of biomarker with the analysed polyherbal formulation and the contents were reanalyzed by the proposed method. Similarly analysed quantity of lupeol in the successive extracts was mixed with the known quantity of biomarker and the contents were reanalysed by the proposed method. Recovery studies were carried out at 80, 100 and 120% levels. The percentage recovery and its %RSD were calculated.

Precision

Precision of the method was validated by

i) Intraday precision

ii) Interday precision

iii) Repeatability

a) Repeatability of measurement

b) Repeatability of sample application

Intraday precision:

Intraday precision was studied by carrying out the analysis of the standard drug at three different concentrations in the linearity range of drug (450, 600 and 750ng/spot) for three times on the same day and % RSD was calculated.

Inter day precision

Inter day precision was studied by carrying out the analysis of the standard drug at three different concentrations in the linearity range of drug (450, 600, and 750ng/spot) for three days over a period of one week and % RSD was calculated.

Repeatability

Repeatability of sample application

Repeatability of sample application was evaluated by spotting 600ng/spot of drug solution six times on pre-coated TLC plate. Plate was then developed, scanned and %RSD was calculated.

Repeatability of measurement

Repeatability of measurement of peak area was evaluated by spotting 600ng/spot of standard drug solutions on pre-coated TLC plate. After development of the plate, the separated spots were scanned without changing position of the plate for six times and %RSD was calculated.

STABILITY STUDIES

When the developed chromatographic plate is exposed to atmosphere, the analytes are likely to decompose. Hence, it is necessary to conduct stability studies.

Stability of the analyte on the plate was studied at different time intervals and peak areas were compared with the peak area of freshly scanned plate.

Stability of the developed plate was found to be 7 hours.

***Invitro* anti-inflammatory activity by *HRBC* membrane stabilization method**

The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture contained 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml *HRBC* suspension[10 % v/v] with 0.5ml of plant extracts of various concentrations (40, 80, 120, 160, 200µg/0.5ml), standard drug ibuprofen in various concentrations (40,80,120,160,200µg/0.5ml) and control [distilled water instead of hypo saline to produce 100 % hemolysis]. The assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the suspension was estimated using spectrophotometer at 560nm. The percentage of hemolysis produced in the presence of distilled water was taken as 100 %. The percentage of HRBC(Human red blood cell) membrane stabilization or protection was calculated using the formula,

Percentage stabilization = 100 – [(optical density of drug) ÷ (optical density of control) × 100]

RESULTS AND DISCUSSION

Preliminary phytochemical studies revealed the presence of triterpenoids, tannins and glycosides in successive extracts. The extract of methanol showed more number of spots when compared to other extracts in the preliminary TLC studies using toluene: methanol [9:1,%v/v] as the mobile phase. The percentage yield during extraction was highest in diethyl amine extract which was found to be 2.446%w/w.

HPTLC METHOD

For the determination of lupeol by HPTLC method different mobile phase systems were tried. A system comprising of toluene: methanol (9:1,%v/v) was selected because this system was foumd to give good separation with symmetric peaks, (Rf value: 0.49 ± 0.03) at a selected wavelength of 366 nm. Calibration curves were drawn with peak areas of standard drug versus concentration. Linearity was found over the concentration range of 300 to 900ng/spot (r=0.9982). After the development, the plate was stable up to 7 hours. Low relative Standard Deviation value showed that the developed method is precise. Limit of detection was found to be 50ng/spot and limit of quantification was found to be 300ng/spot. Results showed that more amount of lupeol was present in petroleum ether extract when compare to other successive extracts. Recovery study was carried out at 80%, 100% and 120% levels. Good recovery values showed that the method is free from interferences. This method was successfully used for the determination of lupeol from polyherbal formulation.

***Invitro* anti-inflammatory activity**

The anti -inflammatory activity of extracts of *Crataeva nurvala* bark and its polyherbal formulation was studied by *HRBC* membrane stabilization method. The results showed that the polyherbal formulation and medicinal plants containing lupeol as the active constituent may be utilized for their anti-inflammatory properties.

Table 1: Analysis of successive extracts

Successive extracts	Amount of lupeol present in 10 mg of extracts	%RSD*
Petroleum ether	1.28 mg	0.5231
Diethyl amine	0.65 mg	0.6543
Methanol	0.17 mg	0.4531

***Mean RSD of six observation**

HPTLC finger prints of *Crataeva nurvala* plant extracts

Fig.1: Fingerprinting of petroleum ether extract

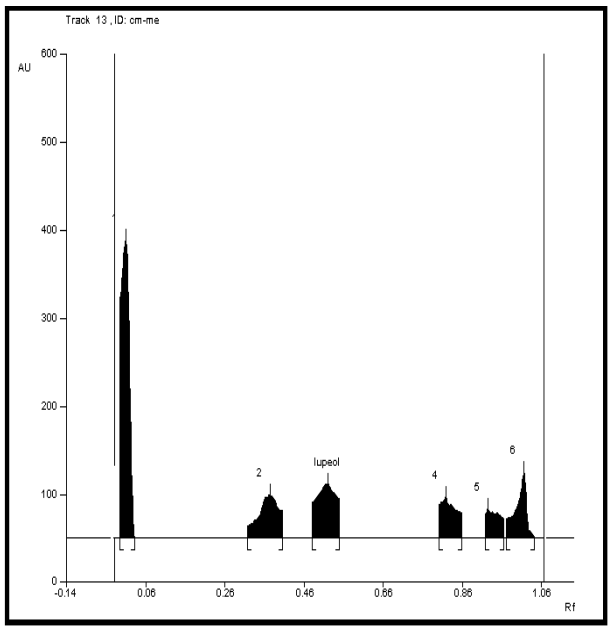

Fig.2: Fingerprinting of diethyl amine extract

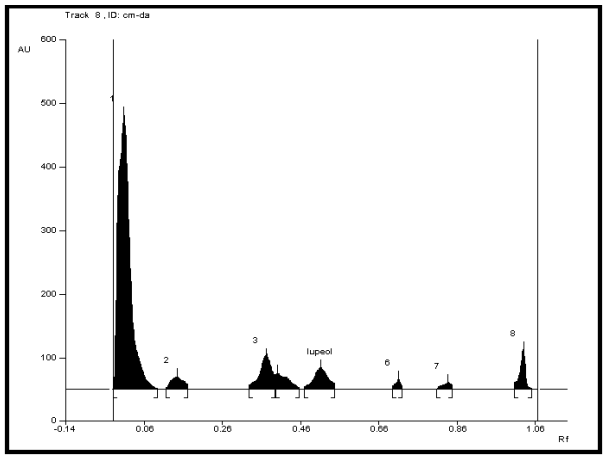

Fig.3: Fingerprinting of methanol extract

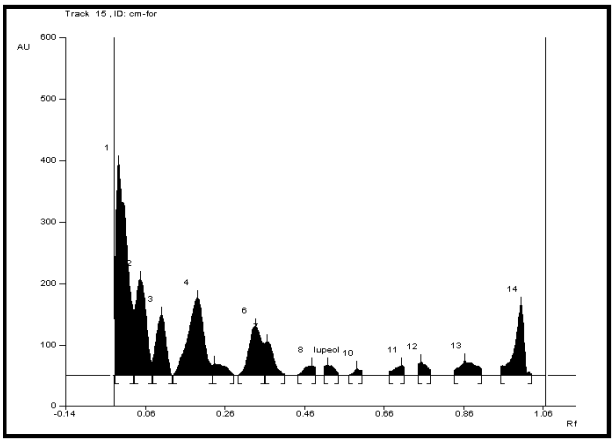

Table 2: Analysis of polyherbal formulation

Polyherbal formulation	Amount of lupeol present in 10 mg of extract	%RSD*
Himplasia	0.73 mg	0.5431

***Mean RSD of six observation**

Table 3: Calibration data of lupeol (300-900 ng/spot)

Concentration(ng/spot)	Peak area
300	531.08
450	694.54
600	830.33
750	1031.03
900	1195.15

Fig.4: Fingerprinting of polyherbal formulation

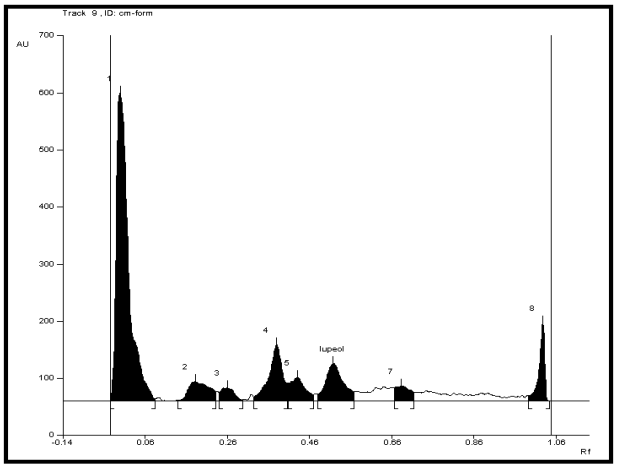

Fig 4: Calibration graph of lupeol (300-900 ng/spot)

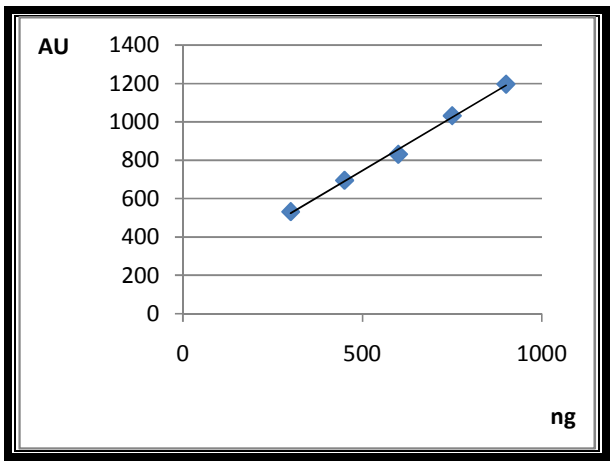

Table 4: Validation studies:

Validation parameters	Concentration(ng/spot)	%RSD
Intraday precision	450	1.158
	600	0.819
	750	0.727
Interday precision	450	1.64
	600	1.26
	750	1.69
Repeatability of sample injection	600	1.122
Repeatability of measurement	600	1.19

Table 5: *HRBC* MEMBRANE STABILISATION ACTIVITY Control absorbance [Ac]: 0.9471

Sample	Concentration (µg/ml)	Absorbance at 560 nm [As]	%HRBC membrane stabilisation (%)	IC_{50} (µg/ml)
Petroleum ether Extract	40	0.8219	13.21	159.83
	80	0.7614	19.60	
	120	0.6100	35.59	
	160	0.4714	50.22	
	200	0.3874	62.56	
Diethyl amine Extract	40	0.8416	11.13	181.75
	80	0.7819	17.44	
	120	0.6431	32.09	
	160	0.4926	47.98	
	200	0.4418	53.35	
Methanol Extract	40	0.8105	14.42	183.32
	80	0.7662	19.10	
	120	0.6941	26.71	
	160	0.5210	44.98	
	200	0.4619	57.90	
Polyherbal formulation Extract	40	0.8498	10.32	168.36
	80	0.7754	16.76	
	120	0.6571	31.23	
	160	0.5064	48.49	
	200	0.4109	59.76	
Standard [Ibuprofen]	40	0.7562	20.15	119.21
	80	0.5931	37.37	
	120	0.4671	50.68	
	160	0.3915	58.66	
	200	0.2712	71.36	

CONCLUSION

The anti-inflammatory activity was highest in petroleum ether extract when compared to other extracts. The bark of *Crataeva nurvala* contain high amount of triterpenoids, which is responsible for the main pharmacological actions like anti-oxidant, diuretic, anti-inflammatory actions, etc. Therefore, the bark of *Crataeva nurvala* was selected for the present work. Many Ayurvedic formulations contain *Crataeva nurvala* bark powder or the extract of the bark powder as the main active portion for the therapeutic action. One polyherbal formulation containing *Crataeva nurvala* were selected for the current study. Good recovery values showed that the HPTLC method is free from interferences. This method was successfully used for the determination of lupeol from polyherbal formulation. The anti-inflammatory activity was highest in petroleum ether extract when compared to other extracts.

ACKNOWLEDGEMENT

The authors are thankful to SNR Sons Charitable Trust, Coimbatore 641044, Tamilnadu for providing adequate facilities in our institution for carrying out this work.

REFERENCES

1. Sethi PD. Quantitative Analysis Of Drugs in Pharmaceutical Formulations. 3rd ed. Mumbai: CBS Publishers and Distributors; 1997. p.1-56.
2. Nishritha. B, Sanjay. S Invitro regeneration of clonally uniform plants of crataeva magna: a high value medicinal tree by axillary branching method. New Forests. 2009;38(1)53-65.
3. Harwood L M, Moody C J. Experimental organic chemistry: Principles and Practice; Wiley Blackwell publishers; (Illustrated edition). p.122-125.
4. Agrawal S.S, Paridhavi. Herbal drug technology. P.324-326
5. Sanayaima RK, Kaur A, Agrawal A, Babbar SB. Cryopreservation of in vitro-grown shoot tips of *Crateva nurvala* Buch. Ham, an important medicinal tree. Cryoletters 2006;27(6):375-386
6. Walia N, Kaur A, Babbar SB. An efficient, in vitro cyclic production of shoots from adult trees of *Crataeva nurvala* Buch. Ham. Plant Cell Rep 2007;26(3):277-284
7. Martini LH, Jung F, Soares FA, Rotta LN, Vendite DA, Frizzo ME, et.al Naturally occurring compounds affect glutamatergic neurotransmission in rat brain. Neurochem Res 2007;32(11):1950-1956
8. Parimala S., Kuamr V.H., Kumar S.M.S., Suthakaran R., Selvan A.T., "Analgesic and CNS depressant Activity of Methanolic Extracts of Cissus Vitegenia And Cissus Pallida", Int. J. Res. Dev. Pharm. L. Sci., 2012, 2 (2), pp. 330-332.
9. Kumari A, Kakkar P. Screening of antioxidant potential of selected barks of Indian medicinal plants by multiple *invitro* assays. Biomedical and Environmental Sciences 2008;21(1):24-29
10. Babbar SB, Walia N, Kaur A. Large scale *invitro* multiplication of *Crataeva nurvala* Methods Mol Biol 2009;547:61-70
11. Gagandeep, Meera, Kalidhar SB. Chemical Investigation of *Crataeva nurvala* Buch. Ham. Fruits.Indian Journal of Pharmaceutical science 2009;71(2)129-130
12. Pulok K.Mukherjee. Quality control of herbal drugs; second edition 2002; Business Horizons Pharmaceutical Publishers, P.405-406
13. Soxhlet, F. Die gewichtsanalytische Bestimmung des Milchfettes, Polytechnisches J. (Dingler's) 1879, 232, 461.
14. Agrawal S.A., "Evaluation of Anti Ulcer Activity of Oldenlandia Corymbosa (L)", Int. J. Res. Dev. Pharm. L. Sci., 2012, 2 (2), pp. 363-367.
15. Pulok K.Mukherjee. Quality control of herbal drugs; second edition 2002; Business Horizons Pharmaceutical Publishers, chapter-13 HPTLC P.493-517

How to cite this article:

Jayaprakasam R., Velayutham S., jyothi B. A., Philips N., Ravi T. K., "Quantitative estimation of lupeol in polyherbal formulation and successive extracts of *crataeva nurvala* and their *in vitro* anti-inflammatory activity study", Int. J. Res. Dev. Pharm. L. Sci., 2013, 2(6), pp. 699-704.

