Int J Pharm Bio Sci 2013 Apr; 4(2): (P) 532 - 537

Research Article **Natural Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

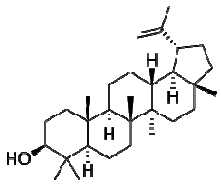
ISOLATION OF LUPEOL, HPLC QUANTIFICATION AND ITS ANTI-HIV POTENTIALS

*** EKTA MENGHANI[1] AND SITA KUMARI SHARMA[2]**

[1]*Department of Biotechnology, Mahatma Gandhi Institute of Applied Sciences, JECRC University, Jaipur, Rajasthan, India.*
[2] *Department of Biotechnology Suresh Gyan Vihar University, Jaipur, Rajasthan, India.*

ABSTRACT

Throughout human history natural products have been used as remedies to cure or treat various diseases. Even traditionally it has been surpassed by the amazing technology and pharmaceutical development that emerged with the promise of early healing. Humans continue to be affected by various diseases, mainly due to natural forces such as drug resistant microbes and insects. Consequently, an imperative need exists to connect the ethno pharmacological information with the newest drug discovery new active natural metabolites. Hence, it an urgent need to screen and isolate new natural products from plant sources and search for novel leads as therapeutic targets for future. Further, novel isolates and active fractions will also be part of active diet to enhance the health and lifestyle. Therefore, in present work attempts were made to search for new isolates with potential efficaciesviz. antioxidant, chemo preventive , cardio preventive, cardio protective and dietary supplement, among these our research attempts were made to isolation of lupeol from *D. rubrum* and its quantification. Lupeol exhibits a broad spectrum of biological activities and can be used as chemo preventive to avoid several diseases. Screening of lupeol suggested that it has positive potentials as anti HIV drug.



KEYWORDS*: Lupeol, Anti HIV Agent, Quantification*

EKTA MENGHANI
Department of Biotechnology, Mahatma Gandhi Institute of Applied Sciences, JECRC University, Jaipur, Rajasthan, India.

**Corresponding author*

INTRODUCTION

HIV (Human immune deficiency type 1) infections are epidemic underway. The world is at the edge of degradation of environmental conditions and pollutions, its time to search for bioactives that have SAR similar to AZT and that work as specific inhibitors of the viral encoded reverse transcriptase (RT). Most rational approach till date is chemotherapy and proves to be most effective for suppression of viral infection. Whereas medicinal plant is any plant in which one or more of its part contains substances that can be used for therapeutic purposes or which are precursors for chemo pharmaceutical semi synthesis. Plants have been used in traditional medicine for several thousand years [1]. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine [2]. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world [3, 4,5,6,7.] Documenting the indigenous knowledge through ethno botanical studies is important for the conservation and utilization of biological resources. Today according to the World Health Organization (WHO), as many as 80% of the world's people depends on traditional medicine for their primary healthcare needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases [8]. Due to less communication means, poverty, ignorance and unavailability of modern health facilities, most people especially rural people are still forced to practice traditional medicines for their common day ailments. Most of these people form the poorest link in the trade of medicinal plants [9]. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [10].In the developed countries, 25 per cent of the medical drugs are based on plants and their derivatives [11]. A group of World Health Organization (WHO) experts, who met in Congo Brazzaville in 1976, sought to define traditional African medicine as 'the sum total of practices, measures, ingredients and procedures of all kinds whether material or not, which from time immemorial has enabled the African to guard against diseases, to alleviate his/her suffering and to cure him/herself' [12]. Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future 2. Plants are by far the most important source of natural therapeutics, and their role in enhancing the longevity and quality of life is gaining prominence throughout the world and still the plant is often the most neglected part of plant-based medicines. Although, millions of consumers purchase medicinal plant preparations on the basis of anecdotal and scientific evidence of efficacy but very little is known about the factors that make medicinal plants different from other species. Current problems with medicinal plant products that compromise the quality and safety of medicinal plant products have included contamination with biological and environmental pollutants, adulteration with misidentified species, and the unsustainable harvest resulting in quantitative and qualitative variations in bioactive compounds. It is, therefore, necessary to standardize the medicinal plants widely used throughout the world. In view of the current importance of and interest in herbal drugs, it is necessary to prepare an International Codex containing the details of such plants so that their sale and utilization could be controlled judiciously. *Piper* species are known to be a rich source of *Piper* amides and their derivatives, as a result of which the plant species carry potent pharmaceutical properties like: diuretic, carminative, stimulant, etc. [13, 14, 15, 16.The] prime objective of this work is to study and set up certain fundamental diagnostic standards for the identification and authentication of a few

important drugs such as *D. rubrum* used in the Ayurvedic System of Medicine as a substitute to *P. zeylanica*. In this, bioactivity guided fractionation was performed for isolation of lupeol which exhibits a broad spectrum of biological activities and can be used as chemo preventive to avoid several diseases. Screening of lupeol suggested that it has positive potentials as anti HIV drug.

MATERIALS AND METHODS

Plant material

Samples of *D. rubrum* was collected from various pockets of Mt. Abu and Sirohi region of Rajasthan in the month of August 2009 and used in the study.

HPLC Analysis

The HPLC analysis was performed using a Shimadzu Model-VP 135P2 equipped with a UV spectrophotometric detector set at 254 nm, column: Luna 5µ C_{18} (2) 100Å (250 x 4.6 mm; 5 particle diameter), flow rate: 1ml/min, injection volume 20 µl in benzene (HPLC grade).

Extraction and isolation

The whole plant of *D. rubrum* was individually extracted with ethanol for 36 hrs, filtered and concentrated to dryness. Later from each, 10 mg extract of *D. rubrum* was dissolved in 5 ml benzene separately and used for HPLC analysis.

Quantification of lupeol in D.rubrum by HPLC

Pet. ether extract of *D. rubrum* (terpenoid rich fraction) was weighed (10, 20, 50 and 100 mg) and dissolved in 10 ml benzene (hplc grade) to prepare a concentration of 1, 2, 5 and 10 mg/ml. 200 µl of each concentrations of *D. rubrum* was injected onto HPLC and the peak which appeared at the same retention time as that of standard lupeol **(I)** was recorded. This value was used to calculate the amount of **I** in the extract by using the linear equation obtained from the composite standard curve. The reproducibility of quantitative analysis was verified by carrying out three replicate injections of each extract and coefficient of variation for each determination was calculated. In the present work, various calculations were achieved by Pearson's correlation formula, which is otherwise used in many forms for correlation co-efficient (*r*) and co-efficient of variation (cv):

$$r = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{N}}{\sqrt{\left(\Sigma X^2 - \frac{(\Sigma X)^2}{N}\right)\left(\Sigma Y^2 - \frac{(\Sigma Y)^2}{N}\right)}} \qquad cv = \frac{\sigma}{x} \times 100$$

Composite standard curve

The area of corresponding lupeol peak and concentration in D*. rubrum* were plotted as a composite standard curve.

Antiviral assay [17]**.**

Cytotoxicity assays in lymphocytes [18].

RESULTS

The quantitative evaluation of adjoining elution curves was done by calculating the resolution ($R = 2\omega t/w_a+w_b$), where ωt is the difference between peak of interest and preceding peak and w_a and w_b are the width of peaks respectively. An easier interpretation of the HPLC tracing, as obtained in this study, was achieved when the peak area was divided by the area of reference peak and the retention time (rt) was plotted against the respective peak

area gave histograms as "normalized fingerprints".In the present investigations, attempts have been made to evaluate various extracts and generate some "fingerprints as markers". In *D. rubrum*, HPLC chromatograms showed different retention time and peak area, which are characterized as "fingerprints". The variation in the lupeol concentration can be used to compare the genuine samples, and thus an efficient marker in identification in quality control of a drug.HPLC chromatograms of extract of *D. rubrum* exhibited lupeol at rt 7.0 min. and others (Figure 1).

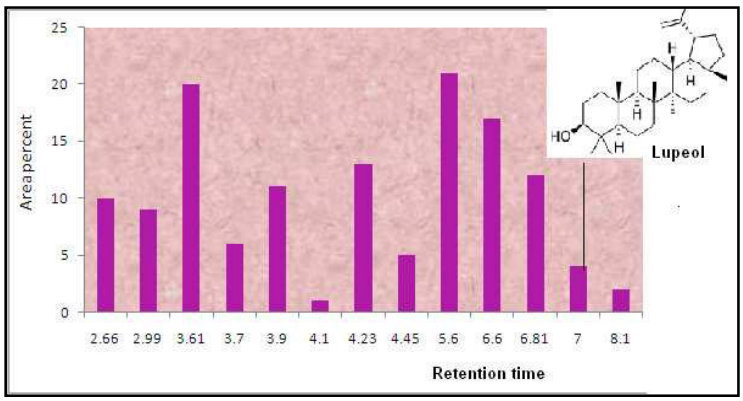


Figure 1
HPLC Chromatograms normalized fingerprints of pet. Ether extract D. rubrum

HPLC analysis of pet. Ether extract of *D. rubrum*, exhibited a prominent peak of lupeol at rt 7.0 min which was further ascertained by varying the concentration (1, 2, 5 and 10 mg/ml) of the extract, In the assessment of linearity, two calibration curves were plotted in the ranges 1.0 –5.0 and 5.0-10.0 mg/ml (Fig. 2). Three replicates of each range were analyzed. The assay value of lupeol was found to be 0.357%. The correlation coefficients for standard curves were 0.9933 and 0.9997. Standard deviation 8.38% and the coefficient of variation (CV) among the two curves was 5.77%. Validation of analytical method exhibited the CV of analysis less than 6%. The composite linear equations obtained from the regression analysis was y = 969.1x + 327.81, R^2 = 0.9992 where y is the area of **I** and x is the amount of the extract injected (Figure 2).

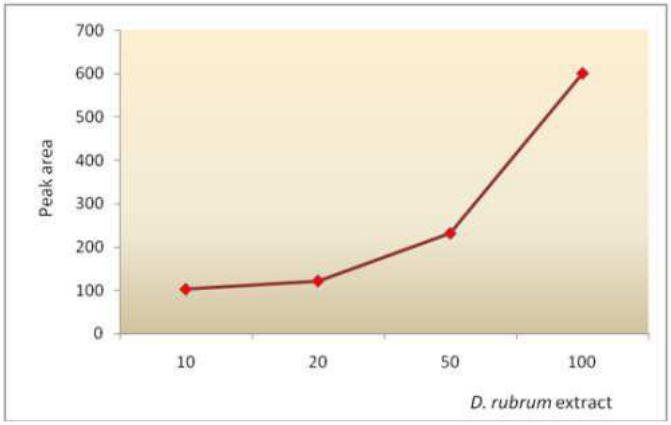


Figure 2
The Composite standard calibration curve for quantification of lupeol in D. rubrum by HPLC

Table 1
Anti-HIV screening of lupeol and its cyto-toxic assay

Anti-HIV-1 Activity in human PBM cells						Cytotoxicity[b] (IC_{50}, µM) in:		
Code	NBK #	EC_{50}, µg/ml	EC_{90}, µg/ml	Slope	R^2	PBM	CEM	VERO
EM-008*	H	≥ 10 (46.7) > 10 (36.5)				n/a	n/a	n/a

The extract and active compounds showed appreciable activity against HIV-1/LA1 (EC_{50} < 10 µM or < 10 µg/mL). The HIV drug susceptibility assay was done as described in Schinazi et al., 1990 and Cytotoxicity assays in PBM, CEM and Vero cells were done as previously in Stuyver et al., 2002. Fraction had pink wells during supernatant harvesting indicating they may be toxic. Showing that it is partially soluble in DMSO. Further, toxicity screening will be performed and addition of novel proteins to reduce the toxicity and generation of novel therapeutics for future generations.

CONCLUSION

Lupeol possessed appreciable activity while antiviral assays in human peripheral blood mononuclear cells (PBMCs) and as bioactive is more effective than pet. Ether extract of *D. rubrum,* further prove the structure possessed the activity. The lupeol possessed hydroxyl group at 3rd position and pent acyclic ring at C22 which will aid and prove SAR and positive therapeutic. If there is an adjoining of some sugar at 3rd position to reduce toxicity and increase efficacy. In summary, results of the present *D. rubrum* extract and it's bioactive in both the antiviral and Cytotoxicity studies indicate that these are highly potent compound and are worthy for pharmacological investigation to determine their effectiveness in animal retroviral models. The aim is for generation of highly potent and less toxic bioactive from natural origin.

ACKNOWLEDGMENT

Authors feel pleasure to give special thanks to Prof. Raymound Schinazi and his co-workers for screening and providing the data of Anti-HIV Activity on Indian Medicinal Plants and their bioactives. Further, Authors also acknowledge the Department of Science and Technology, Jaipur for providing financial support under the banner of Centre with potentials for Excellence in Biotechnology to MGIAS, JECRC Campus, Jaipur.

REFERENCES

1. Abu-Rabia A: Urinary diseases and ethnobotany among pastoral nomads in the Middle East.Journal of Ethnobiology and Ethnomedicine 2005. 1:4: doi: 10.1186/1746-4269-1-4.
2. Pei SJ: Ethnobotanical approaches of traditional medicine srudies: Some experiences from Asia. Pharmaceutical Biology 2001, 39:74-79.
3. Lev E: Ethno-diversity within current ethno-pharmacology as part of Israeli traditional medicine – A review. Journal of Ethnobiology and ethnomedicine 2006., 2:4:
4. Gazzaneo LR, Paiva de Lucena RF, Paulino de Albuquerque U: Knowledge and use of medicinal plants by local specialists in an region of Atlantic Forest in the state of Pernambuco (Northeastern Brazil). Journal

of Ethnobiology and Ethnomedicine 2005., 1:9:

5. Al-Qura'n S: Ethnobotanical survey of folk toxic plants in southern part of Jordan. Toxicon 2005, 46:119-126.
6. Hanazaki N, Tamashiro JY, Leitao-Filho H, Gegossi A: Diversity of plant uses in two Caicaras communities from the Atlantic forest coast, Brazil. Biodiversity ands Conservation 2000, 9:597-615.
7. Rossato SC, Leitao-Filho H, Gegossi A: Ethnobotany of Caicaras of the Atlantic forest coast (Brazil). Economic botany 1999, 53:387-395.
8. Khan AU: History of decline and present status of natural tropical thorn forest in Punjab. Pakistan Biological Conservation 2002, 63:210-250.
9. Azaizeh H, Fulder S, Khalil K, Said O: Ethnomedicinal knowledge of local Arab practitioners in the Middle East Region. Fitoterapia 2003, 74:98-108.
10. Diallo D, Hveem B, Mahmoud MA, Berge G, Paulsen BS, Maiga A: An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biology 1999, 37:80-91.
11. Principe P: Monetising the pharmacological benefits of plants.US Environmental protection Agency, Washington, D.C.; 1991.
12. Busia K: Medical provision in Africa – Past and present. Phytotherapy Research 2005, 19:919-923.
13. Charaka Samhita 1949. Vols 4, Shree Gulab Kunverba Ayurvedic Society, Jamnagar, India.
14. Chopra, R.N., I.C. Chopra and B.S. Varma. 1969. Supplement to Glossary of Indian Medicinal Plants, (publication and information directorate, CSIR, New Delhi) p.s 27
15. Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian MedicinalPlants.CSIR, New Delhi. p. 194.
16. Nadkarni, A.K. 1954. *Dr. K.M. Nadkarni's Indian Materia Medica*. Vol. I (pp. 1319) & Vol. II (pp.968) Popular Book Depot, Bombay, India.
17. Schinazi, R. F. Sommadossi, J.P. Saalmann, V., Cannon, D.L. Xie, M. W., Hart,G. C. Smith, G.A. and Hahn, E. F. Activity of 3'-azido-3'-deoxythymidine Nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 34 (6):1061-1067, 1990.
18. Stuyver, L.J., Lostia, S., Adams, M., Mathew, J., Pai, B.S., Grier, J., Tharnish, P., Choi, Y., Chong, Y., Choo, H., Chu, C.K., Otto, M.J., Schinazi, R.F. Antiviral activities and cellular toxicities of modified 2',3'-dideoxy-2',3'- didehydrocytidine analogues Antimicrob. Agents Chemother. 46 (12):3854-3860, 2002.