ANTIRETROVIRAL ACTIVITY OF NATURALLY OCCURRING AND RELATED SYNTHETIC 1-PHENYLNAPHTHALENE LIGNANS

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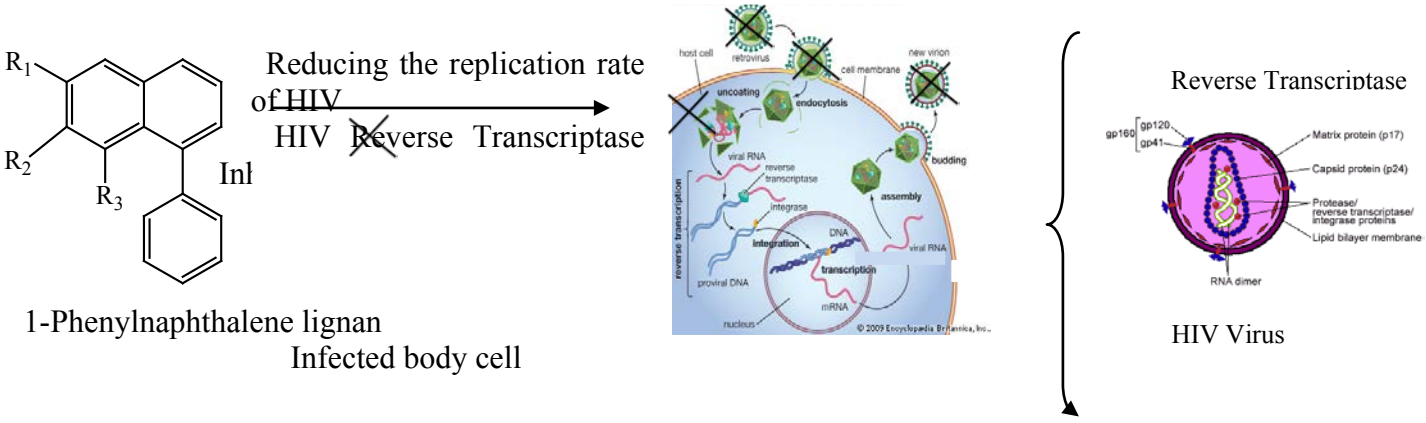
Abstract

Anti-HIV agents are urgently required due to global and widespread infection of HIV/aids. Most of the anti HIV agents are nucleosides but besides the high cost there are adverse effects and limitations associated with chemotherapy applied. Biodiversity of plant kingdom has always provided a source of new drug candidates for almost all disease areas.1-Phenylnaphthalene and Pericarbonyl lactone lignans and their synthetic derivatives proved as potent immunomodulators, were further tested for the Reverse Transcriptase (RTase) inhibition activity by the Reverse Transcriptase Assay, Colorimetric. For the experimental studies, two plants- Ruta graveolens and Jatropha gossypifolia have been chosen containing the lignans showing structural similarity with the synthetic compounds. The study revealed that the naturally occurring and related synthetic organic compounds with 1-Phenylnaphthalene system were active inhibitors of HIV RTase and inhibited the 50 % RTase activity in the concentration range of 20-48 µg/ml. Thus the present investigation can fulfill our aim of HIV treatment, where 1-Phenylnaphthalene lignans can assist the immune system in driving out the HIV virus and are also expected to lower the incidence of numerous opportunistic infections.

Key words - Antiretroviral activity, 1-Phenylnaphthalene lignans, Reverse Transcriptase Assay, HIV Reverse Transcriptase inhibition activity, Plant extract, Ruta graveolens, Jatropha gossypifolia.



1 INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is a formidable pandemic that is still wreaking havoc worldwide. The causative moiety of the disease is human immunodeficiency virus (HIV).[1] The viral enzyme, reverse transcriptase (RTase) catalyses the formation of proviral DNA from viral RNA, the key stage in viral replication. Its central role in viral replication makes RTase a prime target for anti-HIV

therapy.[2] In AIDS, the immune system is devastated and collapsed. Most of the antiretroviral drugs are limited in use owing to their severe toxicity, adverse effect and emergence of drug resistance.[3-5] This has driven many scientists to look for new antiretrovirals with better efficiency, safety and affordability.

A phytotherapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants.[6-8] In our recent studies, synthetic and natural 1-Phenylnaphthalene lignans have been proved as potent immunomodulatory agents.[9,10] To utilize their immunomodulatory potential as well, we planned to test them for the antiretroviral (RTase inhibitory) activity by Reverse Transcriptase Assay,Colorimetric.[11-13] The assay has many advantages : safe (avoids the use of radioisotopes), sensitive, reliable (accurate and reproducible), convenient (allows for large scale RT inhibitor screening), fast (results in 4 h) and easy (follows a standard ELISA protocol). Eight synthetic derivatives were prepared in the laboratory whereas the study of natural Phenylnaphthalene lignans was carried out by choosing two popular medicinal plants - *Ruta graveolens* and *Jatropha gossypifolia* consisting of similar lignans.14-16 When evaluated for their cytotoxicities against HIV RTase, all of the synthetic compounds and plant extract samples were found to be potent HIV inhibitors. They have potential to interfere with particular viral target, which can result in mechanisms of action complementary to those of existing antiretroviral drugs.

The objective of the present study was to discover a new approach in which human immunodeficiency virus (HIV) can be eradicated from an infected individual by intensified antiretroviral treatment coupled with immunomodulation. HIV treatment based on immunostimulation will improve the function of the immune system of patients; can make the immune system competent in driving out the virus from the reservoirs.

2 MATERIALS AND METHOD

2.1 Synthetic 1- Phenylnaphthalene derivatives

Perkin condensation of aromatic aldehydes with β-benzoyl propionic acid gives α-arylidine-γ-phenyl-Δ,β,γ-butenolides.[17] The butenolides were cleaved with alcoholic sodium carbonate to afford α-arylidine-β-benzoyl propionic acid.[18] This keto acid was then treated with different reagents like CH_2N_2, formaline to get various derivatives. Cyclization of α-arylidene-β-benzoyl propionic acid and its derivatives ultimately exhibited to 1-Phenylnaphthalene and Pericarbonyl lactone lignans.[19, 20]

2.2 Plant material & Extraction Methodology

Ruta graveolens (L) and *Jatropha gossypifolia* (L) collected from Shree Shail Medifarm, plant nursery, Nagpur and authenticated from the Department of Botany, RTMNU, Nagpur. The specimen voucher number is 9605 & 9606 for *Ruta* and *Jatropha* respectively.

The whole plant materials were shade dried and powdered separately with mechanical blender. About 800 grams of fine powder of each plant sample was prepared and kept in separate air tight containers.

The plant material of *Ruta graveolens* (about 750g) was defatted with petroleum ether (60 – 80^{o}C) and extracted with methanol for 24 hours in a Soxhlet extractor ; whereas *Jatropha gossypifolia* (about 500g) was exhaustively extracted with petroleum ether for about 30 – 35 complete cycles. After extraction, solution obtained was evaporated at 45°C under reduced pressure till a viscous mass material was obtained. The dried methanolic extract (ME) of *R. graveolens* and petroleum ether extract (PE) of *J. gossypifolia* were stored in airtight containers and placed in a refrigerator. The ME and PE were used for the experimental study.

2.3 Reverse Transcriptase Assay

The Reverse Transcriptase Assay (RTase assay kit by Roche chemicals, Germany), is a colorimetric method for the quantitative determination of RT activity in samples. The reverse transcriptase assay, colorimetric takes advantage of the reverse transcriptase to synthesize DNA starting from the template/primer hybrid poly (A) × oligo $(dT)_{15}$.

2.3.1 Test Principle

Biotin and digoxigenin-labeled DNA binds to the surface of microplate (MP) modules that have been precoated with streptavidin. In the next step,an antibody to digoxigenin, conjugated to peroxidase (anti-DIG-POD) binds to the digoxigenin-labeled DNA. In the final step, the peroxidase substrate ABTS is added. The peroxidase enzyme catalyses the cleavage of the substrate, producing a colored reaction product. The absorbance of the samples can be

determined using a microplate (ELISA) reader [21] and is directly correlated to the level of RT activity in the sample.

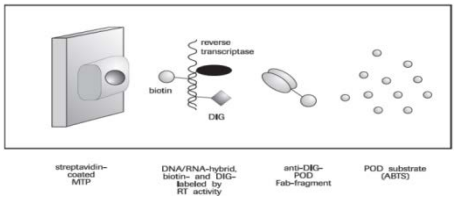


Figure 1. Test principle of Reverse Transcriptase Assay

2.3.2 Kit contents

1. **HIV-1 reverse transcriptase** - Recombinant HIV-1-RT, lyophilizate, from potassium phosphate buffer, pH 7.4 containing 0.2% bovine serum albumin (BSA).
2. **Lysis buffer**(ready to use) - Tris- buffer (50 mM Tris,80 mM potassium chloride,2.5 mM DTT, 0.75 mM EDTA and 0.5% Triton X-100,pH 7.8).
3. **Reaction mixture** (ready to use) - 46 mM Tris-HCl, 266 mM potassium chloride, 27.5 mM magnesium chloride, 9.2 mM DDT, 10 µM dUTP/dTTP, template/primer hybrid.
4. **Microplate (MP) modules** (8-wells)- Precoated with streptavidin and post coated with blocking reagent, shrink-wrapped, with a desiccant capsule. 7 bags containing 4 MP modules (total of 224 wells per kit).
5. **Anti-digoxigenin-peroxidase** (Anti-DIG-POD) - Polyclonal anti-body from sheep, lyophilizate.
6. **Cover foil**- Used to avoid evaporation.
7. **Washing buffer** (ready to use) - 225 ml of autoclaved redist. water per bottle -7 of Assay kit.
8. **Substrate buffer**-Sodium perborate and citric acid / phosphate buffer.
9. **ABTS Substrate –**tablets.
10. **ABTS Substrate solution**- One ABTS tablet in 5 ml substrate buffer , mixed by stirring.

3 EXPERIMENTAL PROCEDURE

- 4-6 ng recombinant HIV-1-RT was diluted in lysis buffer (20 µl /well) in a separate reaction tube. Lysis buffer with no HIV-1-RT added was used as a negative control. 20 µl of Test compounds (lignan derivatives or *Ruta graveolens* extract (MeOH) or *Jatropha gossypifolia* extract (Petroleum ether); 10 – 50 µg/ml) diluted in lysis buffer and 20 µl reaction mixtures were added per reaction tube and incubated for 1 h at 37°C.
- Enough foil bags were opened for the number of MP modules to be used.
- The samples (60 µl) were transferred into the wells of the MP modules, covered with a cover foil and incubated for 1 h at 37°C.
- The solution was removed completely and rinsed 5 times with 250 µl of washing buffer per well for 30 s each and washing buffer was removed.
- 200 µl of anti-DIG-POD (200 mU/ml) per well was added, covered with cover foil and incubated again for 1 h at 37°C.
- The solution was removed completely and rinsed 5 times with 250 µl of washing buffer per well for 30 s each and washing buffer was removed.
- 200 µl of ABTS substrate solution was added per well and incubated at +15 to +25°C until the green color is developed (Figure 2).
- Absorbance of the samples was measured at 405 nm (reference wavelength: 490 nm) using a micro plate (ELISA) reader.
- **Interpretation:** The resulting signal intensity is directly proportional to the actual RT activity. Reverse Transcriptase concentration of unknown samples can be determined by plotting the observed absorbance values on the y-axis, extrapolating to meet the calibration curve (Figure 3) and reading the RT concentration from the x-axis.

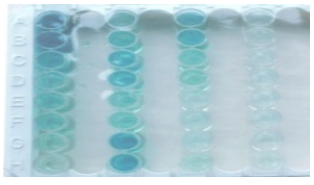


Figure 2. RTase inhibition colour reaction in streptavidin coated microplates

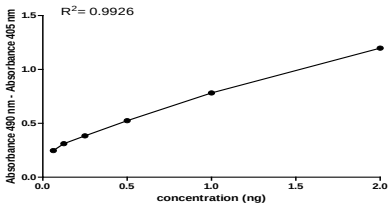


Figure 3. Concentration response (calibration) for RTase colour reaction

4 RESULT AND DISCUSSION

4.1 Synthetic Lignans

Synthetic derivatives of 1-Phenylnaphthalene lignans were tested for their *in-vitro* quantitative determination of HIV Reverse Transcriptase inhibition activity using Reverse Transcriptase Assay, Colorimetric. As shown in Figure 4 and depicted in Table 1, all the synthetic compounds inhibited the reverse transcriptase activity. Concentrations required for samples A, B, C, D, E, F and G for 50% inhibition of reverse transcriptase was found to be 48.43 µg/ml, 40.31 µg/ml, 20.85 µg/ml,33.16 µg/ml,44.02 µg/ml,26.39 µg/ml and 34.06 µg/ml respectively. The differential effects of lignans on RTase inhibition may be attributed to the differences in substituents. Amongst the acids and lactones, the derivatives having methylenedioxy substituent i.e 1-Phenyl-6,7-methylenedioxy naphthalene-3-carboxylic acid (C) was found to most potent with the lowest IC_{50} (20.85 µg/ml) followedby1-Phenyl-6,7-methylenedioxy naphthalene lactone (F) with IC_{50} (26.39 µg/ml).

4.2 Plant Lignans

The two plant extracts when evaluated for antiretroviral properties, maximum activity was facilitated by the methanolic extract of *Ruta graveolens* and petroleum ether extract of *Jatropha gossypifolia.* The reason is given by the phytochemical studies of *Ruta graveolens* [22] and *Jatropha gossypifolia* [23] which showed the presence of similar lignans in these particular extracts. Thus it can be concluded that each plant extract showing RTase inhibition activity is due to presence of 1-Phenylnaphthalide lignans - Helioxanthin in *Ruta graveolens* and Arylnaphthalene in *Jatropha gossypifolia* as shown in Table 1.

Jatropha gossypifolia extract (petroleum ether) and *Ruta graveolens* extract (MeOH) demonstrated the inhibition of RTase activity with IC_{50} value of 47.78 µg/ml and 40.83 µg/ml respectively (Figure 5). Both the plant extracts showed significant inhibition, although the activity at the doses used was less significant as compared to the synthetic lignan compounds. Herbal products symbolize safety and maintain the health and vitality of individuals.

All over findings suggest that both synthetic and naturally occurring 1-Phenylnaphthalene lignans positively inhibits the RTase activity and can be used in the treatment of HIV-infected persons.

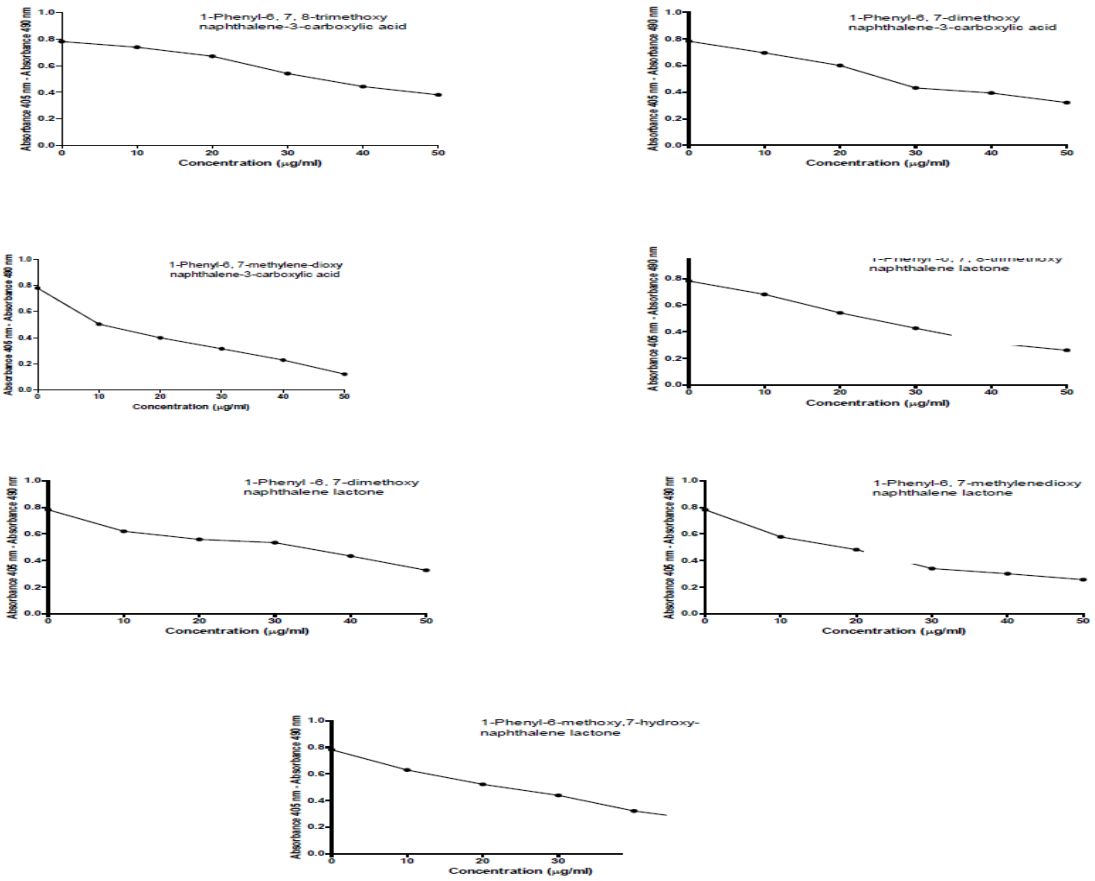
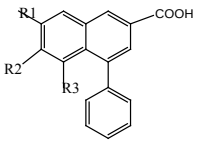
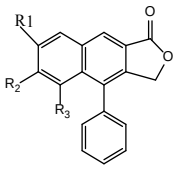
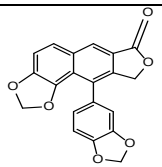
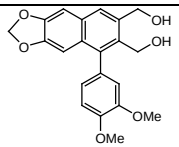


Figure 4. Effect of synthetic lignan derivatives on RTase inhibition

Figure 5. Effect of plant lignans on RTase inhibition

1. Antiretroviral activity with IC_{50} value for the synthetic and natural lignans

	Lignans	**Structure**	**Antiretroviral Activity with IC_{50} values**
Synthetic Compounds	**A-** 1-Phenyl -6,7,8 trimethoxy naphthalene -3 carboxylic acid		48.43 µg/ml
	B- 1-Phenyl -6,7 dimethoxy naphthalene-3 carboxylic acid.		40.31 µg/ml
	C-1-Phenyl-6,7 methylene-dioxy naphthalene -3 carboxylic acid.		20.85 µg/ml
	D- 1-Phenyl -6,7,8 trimethoxy naphthalene lactone		44.02 µg/ml
	E 1-Phenyl -6,7 dimethoxy naphthalene lactone		33.16 µg/ml
	F 1-Phenyl-6,7 methylenedioxy naphthalene lactone		26.39 µg/ml
	G- 1-Phenyl -6 –methoxy,7-hydroxy- naphthalene lactone		34.06 µg/ml
Natural Compounds	**H**-Helioxanthin *(Ruta graveolens MeOH extract)*		47.78 µg/ml
	I- Arylnapthalene lignan *(Jatropha gossypifolia Pet ether extract)*		40.83 µg/ml

A) $R_1 = R_2 = R_3 = OCH_3$
B) $R_1 = R_2 = OCH_3$, $R_3 = H$
C) $R_1 = R_2 = O\text{-}CH_2\text{-}O$, $R_3 = H$
D) $R_1 = R_2 = R_3 = OCH_3$
E) $R_1 = R_2 = OCH_3$, $R_3 = H$
F) $R_1 = R_2 = O\text{-}CH_2\text{-}O$, $R_3 = H$
G) $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$

4 CONCLUSION

In summary, 1-Phenylnaphthalene lignans and their synthetic derivatives are clinical candidates with potential to come up as drugs for treatment of HIV infection. They can clear HIV-like infection by boosting the function of the cells vital to the immune response. In addition, these compounds can be used to prevent tumors secondary to virus infection as well as other infections or disease states that are secondary to the virus infection.

This discussion helps to conclude that in future a fruitful area of future research may be in modifying natural lignans or synthesizing

new lignans with unique structural diversity and potent pharmacological activities.

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REFERENCES

1. Cherman, J.C., Rey, F., Chamaret, S., & Gruest, J., 1983, Science, 220, 868.
2. Gallo, R.C., Salahudin, S.Z., Popovic, M., & Kaplan, M., 1984, Science, 224, 500.
3. Haynes, R.B. 2001, In Improving patient adherence: state of the art, with a special focus on medication taking for cardiovascular disorders. In: "Compliance in Health Care and Research; L.E. Burke, I.S. Ockene, eds., pp 3-21. New York, NY: Futura Publishing Co Inc.
4. Frick, P.A., Gal, P., Lane, T.W., & Sewell, P.C., 1998, AIDS, Patient Care STDS,12, 463.
5. Kalvatchev, Z., Walder, R., & Garzard, D. 1997, Biomed Pharmacother, 51, 176.
6. Min, M., Bae, K.H., Kim, Y.H., Mujashiro, H. & Hattori, M., 1999, Phytother Res,13, 680.
7. Mlinarie, A., Kreft, S., & Umek, A., 2000, Die Pharmazie, 55, 75.
8. Deo, S., Chaudhari, T., & Inam, F., 2012, Int J. Knowl Eng, 3, 143.
9. Deo, S., Chaudhari, T., & Inam, F., 2012, Der Pharma. Chemica, 4, 771.
10.Shih,C.K.,Rose,J.M.,Hansen,G.L.,Wu,J.C.,Bacolla,A., & Griffin, J.A.,1991,Proceedings of the National Academy of Sciences, 88, 9878.
11. Rein, A., Ott, D.E., Mirro, J., Arthur, L.O., Rice, W., & Henderson, L.E., 1993, Proceedings of the National Academy of Sciences, 90, 9721.
12. Ukkonen, P., Korpela, J., Suni, J., & Hedman, K., 1988, Eur J Clin Microbiol Infect Dis, 7, 518.
13.Wu, J.C., Chernow, M., Boehme, R. E., Suttmann, R. T., McRoberts, M. J., Prisbe, E. J., Matthews, T.R., Marx, P.A., Chuang, R. Y., & Chen, M. S., 1988,Antimicrob Agents Chemother, 32, 1887.
14. Tan, C.K., Zhang, J., Li, Z.Y., Tarpley, W.G., Downey, K.M., & So, A.G., 1991, Biochem, 30, 2651.
15. Müller, B., Restle, T., Weiss, S., Gautel, M., & Sczakiel, G. J., 1989, Biol Chem, 264, 13975.
16. Deo, S., Inam, F., Mahashabde, R.P., & Jadhav, A.N., 2010, Asian J Chem, 22, 3362.
17. Avetisyan, A.A., & Tokmadzhyan, G.G., 1987,Chem Heterocycl Comp, 23, 595.
18. Oveden, S.P.B., Yu, J., Wan, S.S., Sberna, G., & Tait, R.M., 2004,Phytochem, 65, 3255.
19. Deo, S., Chaudhari, T., & Inam, F. (February 9-11, 2011) Proceedings of International Conference on Chemistry for Mankind: Innovative Ideas In Life Sciences, 172-179.
20. Walsh C., & Cepko, C.L., 1992, Science, 255, 434-440.
21. Lee, S., Ban, H. S., Kim, Y. P., Kim, B. S., Cho, S. H., Ohuchi, K.,& Shin, K.H., 2005, Phytother Res, 19, 103.
22. Das, B., Banerjee, J., 1988, Phytochem, 27, 3684.

List of abbreviations:-

α = alpha
β = beta
γ = gamma
∂ = delta
CH_2N_2 = Nitrosomethylene
EDTA = Ethylene diamine tetra acetic acid
ng = nanogram
µl = microlitre
µg =microgram