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Evaluation of the immunomodulatory effects of 1-Phenylnaphthalene and Pericarboxyl lactone lignan compounds

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ABSTRACT

Throughout history, natural products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy and biology. Within the sphere of immunomodulation, a number of important new commercialized drugs have been obtained from natural sources, by structural modification of natural compounds or by the synthesis of new compounds, designed following a natural compound as model. To search for improved immunomodulators, 1-Phenylnaphthalene and Pericarboxyl lactone lignans and their synthetic derivatives were tested for the immunomodulatory activity by the assay of Spleen cell proliferation. The investigation found that the naturally occurring and related synthetic organic compounds with 1-Phenylnaphthalene and Pericarboxyl lactone systems were potent in producing immune stimulation. In HIV treatment, they can assist the immune system in driving out the HIV virus and are expected to lower the incidence of opportunistic infections in several immunodeficiencies.

Key words: Immunomodulatory activity, 1-Phenylnaphthalene lignans, Pericarboxyl lactone lignans, spleen cell proliferation, *Ruta graveolens*, *Jatropha gossypifolia*.

INTRODUCTION

The world is gradually turning to herbal formulations which are known to be effective against a large repertoire of diseases and ailments. More importantly, they are not known to cause any notable derogatory effects [1] and are readily available at affordable prices [2]. 1-Phenyl-naphthalenes are versatile moieties in that their pendant like skeleton exists in Pericarboxyl lactone lignans and a number of pharmaceuticals. Pericarboxyl lactone lignans are natural products, important class of biologically active organic compounds and have wide range of therapeutic properties like anti-inflammatory [3], antibacterial [4], antioxidant [5], anti cancer [6] CNS depressants etc [7]. The broad spectrum of biological activities has made them privileged structures in combinatorial drug discovery. The present study was therefore undertaken to explore the immunomodulatory potential of 1-Phenylnaphthalene and Pericarboxyl lactone lignans. The aim of this study is 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. The hypothesis is that eradication is possible only if very potent antiretroviral drugs are delivered in conjunction with an immunomodulatory agent that simultaneously attacks the viral reservoirs [8-10].

Immunomodulation is a therapeutic approach in which we try to intervene in auto regulating processes of the immune system which is known to be involved in the etiology and pathophysiological mechanisms of several diseases [11]. Immunomodulators do not tend to boost immunity, but to normalize it [12]. Part of their benefit appears to be their ability to naturally increase the body's production of messenger molecules, such as cytokines, which mediate and regulate the immune system making it more efficient.

Determination of immunomodulatory activity is carried out by the assay of – spleen cell proliferation [13-14]. Spleen cell proliferation assay is used to evaluate the influence on specific and non-specific measures of immune function. It is one of the most common tools used to evaluate lymphocyte function. The non-specific proliferative response of rat spleen cells has been shown to be depressed in the immunodeficiencies and hence in the present study, cellular immune function was assessed by establishing in vitro spleen cell cultures stimulated with antigen. Eight synthetic derivatives were prepared in the laboratory whereas the study of natural Phenylanthralene lignans was carried out by choosing two popular medicinal plants - *Ruta graveolens* and *Jatropha gossypifolia* consisting of lignans showing structural similarity with the synthetic compounds (scheme 1).

MATERIALS AND METHODS

Synthetic 1- Phenylanthralene derivatives

Perkin condensation of aromatic aldehydes with β -benzoyl propionic acid gives α -arylidene- γ -phenyl- Δ,β,γ -butenolides [15]. The butenolides were cleaved with alcoholic sodium carbonate to afford α -arylidene- β -benzoyl propionic acid [16]. 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-Phenylanthralene and Pericarboxyl lactone lignans [17].

Plant material

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.

Extraction Methodology

The plant material of *Ruta graveolens* (about 750g) was defatted with petroleum ether (60 – 80°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.

Experimental animal

In the experiments, 8 to 10-week old male rats were used. The animals were housed in clean and spacious cages provided with net and feeding bottle, at ambient temperature of 25 ± 2^0 C with 12 hrs. light and 12 hrs dark cycles and provided free access to standard laboratory chow mixture and purified water ad libitum for fixed period so as to acclimatize all animals and to achieve normal constant basal food intake in all.

All experiments were performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSEA) norms.

Proliferation of mice spleen lymphocytes in vitro

Preparation of mice spleen lymphocytes

Single spleen cell suspension was prepared from the spleens of male mice (C57 BL/6), killed by cervical dislocation. Spleens were removed and washed three times with phosphate buffered saline (PBS), cut into small pieces and pressed through a stainless steel mesh (100 mesh) to obtain spleen cell suspension. Suspension was centrifuged (400 \times g, 6 min) and red cells in the spleen cell suspension were lysed with Tris-HCl-NH₄Cl solution (pH 7.2) [18]. Cell suspension was further diluted with 5 Vol of RPMI-1640 medium (Gibco Company (USA) and after mixing and centrifugation, the pelleted cells were resuspended in RPMI-1640 medium to a concentration of 2×10^6 cells/ml.

Spleen cell proliferation assay

Aliquots (180 μ l) of the cell suspension and 20 μ l of synthesized lignan derivatives (50, 100, 200 μ g/ml) or *Ruta graveolens* extract (MeOH) (100, 200, 400 μ g/ml) or *Jatropha gossypifolia* extract (Petroleum ether) (100, 200, 400 μ g/ml) were added to each well of a 96 well plate. PBS and PHA (6 μ g/ml) served as the negative and positive controls, respectively. After incubation at 37°C in a 5 % CO₂ atmosphere for 72 h, 20 μ l Alamar blue reagent (Biosource, Nivelles, Belgium) was added to each well and the incubation continued for another 6 h. Absorption

values at 570 nm (A_{570}) and 600 nm (A_{600}) were measured using a micro enzyme-linked immunosorbent assay (ELISA) autoreader. The proliferation rate (r) was calculated according to the following formula [19]:

$$r (\%) = [117216 \times A_{570} (\text{sample}) - 80856 \times A_{600} (\text{sample})] / [117216 \times A_{570} (\text{control}) - 80856 \times A_{600} (\text{control})] \times 100\%$$

RESULTS AND DISCUSSION

For Synthetic lignans

In the assay of spleen cell proliferation activity, all the synthetic 1-Phenyl-naphthalene compounds significantly stimulated the proliferation of rat spleen lymphocytes at 50 $\mu\text{g/ml}$ dose levels and cell proliferation rates were increased with increased concentrations of synthetic lignan compounds. As shown in Figure (1) and depicted in Table (1), almost 90 % activity was observed at 200 $\mu\text{g/ml}$ concentrations as compared to 6 $\mu\text{g/ml}$ PHA, which served as the positive control. The differential effects of lignans on cell proliferation may be attributed to the differences in structures. From the studies of effects of lignan derivatives on cell proliferation, it is found that, all the 1-Phenyl-naphthalene derivatives showed immunomodulatory activity with a potency to be effective in assumed therapeutic exploitation.

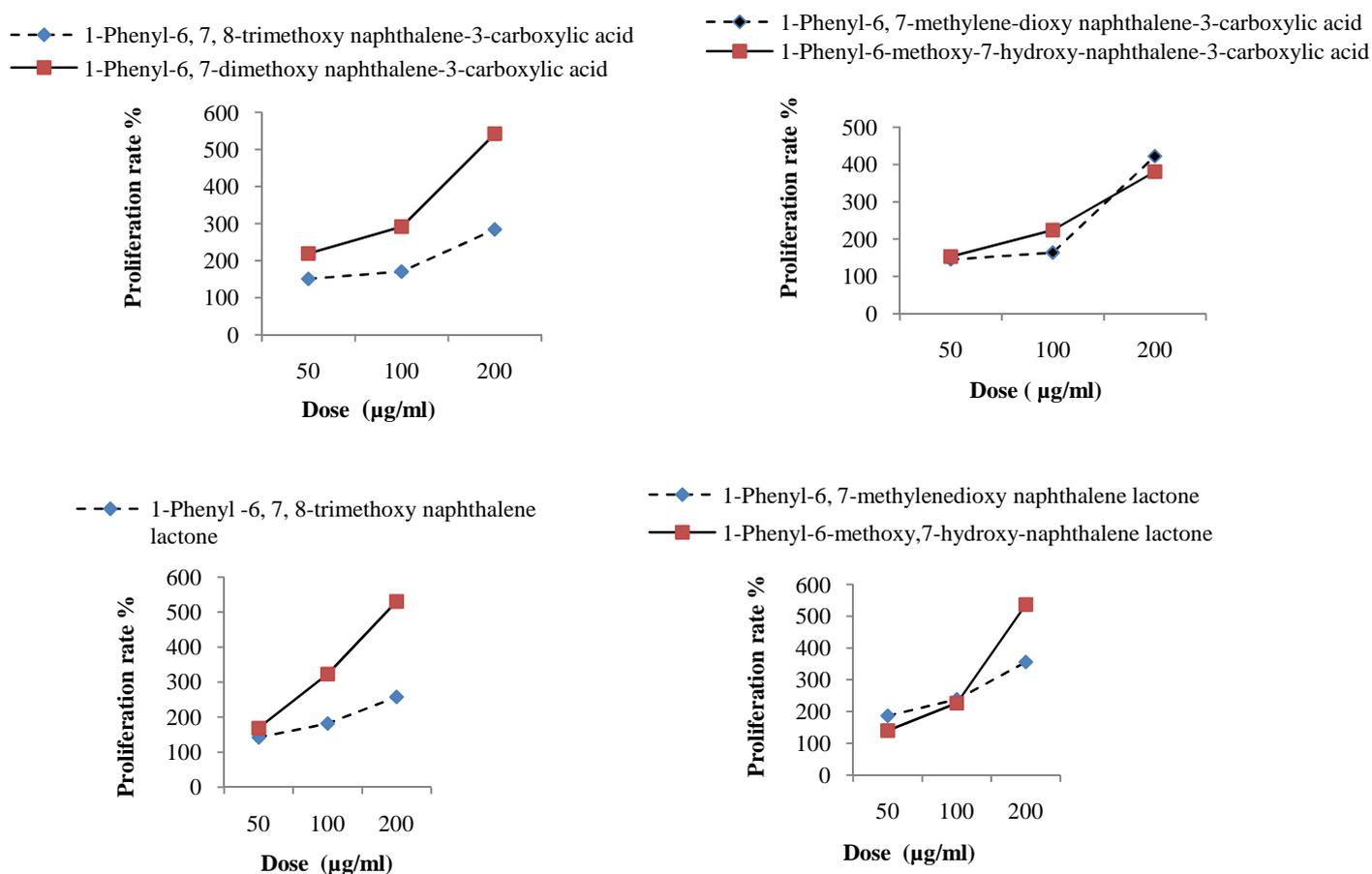


Figure 1: Effect of lignan derivatives on spleen cell proliferation

Plant lignans

The two plant extracts when evaluated for immunomodulatory 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* [20] and *Jatropha gossypifolia* [21] which showed the presence of similar lignans in these particular extracts. Thus it can be concluded that each plant extract showing immunomodulator activity is due to presence of 1-Phenyl-naphthalide lignans - Helioxanthin in *Ruta graveolens* and Arylnaphthalene in *Jatropha gossypifolia* (compound no.9 &10).

The oral administration of the plant extracts at doses of 100,200 and 400 $\mu\text{g/ml}$ significantly accelerated the proliferation of rat spleen lymphocytes when compared to control at 100 $\mu\text{g/ml}$ dose levels and were increased with increased concentration of the extracts as shown in Table 2 & Figure 2. The present observations suggest that both

the herbal extracts can be good sources of immunomodulatory compounds called as lignans. Such an ethno medical approach for diseases is a practical, cost effective and logical for its treatment.

Table 1 Effect of synthesized lignan derivatives on spleen cell proliferation

Sr. No.	Compound	Proliferation rate %		
		50 µg/ml	100 µg/ml	200 µg/ml
1	1-Phenyl-6, 7, 8-trimethoxy naphthalene-3-carboxylic acid	151.141	170.687	284.062
2	1-Phenyl-6, 7-dimethoxy naphthalene-3-carboxylic acid	218.945	291.461	542.715
3	1-Phenyl-6, 7-methylene-dioxy naphthalene-3-carboxylic acid	144.977	163.668	421.949
4	1-Phenyl-6-methoxy-7-hydroxy-naphthalene-3-carboxylic acid	153.117	224.112	380.921
5	1-Phenyl -6, 7, 8-trimethoxy naphthalene lactone	141.658	181.456	257.786
6	1-Phenyl -6, 7-dimethoxy naphthalene lactone	168.316	323.003	530.474
7	1-Phenyl-6, 7-methylenedioxy naphthalene lactone	186.626	238.356	356.391
8	1-Phenyl-6-methoxy,7-hydroxy-naphthalene lactone	139.687	226.573	537.18
9	PHA (6 µg/ml)	617.26		
10	PBS	129.64		

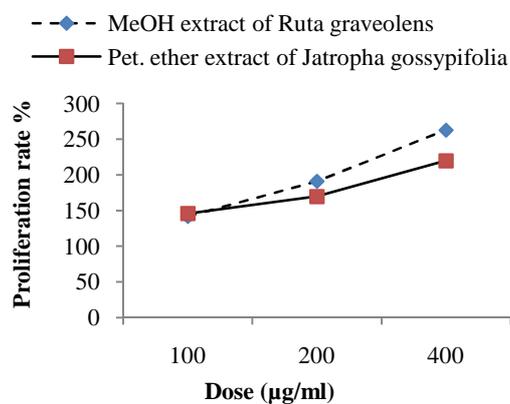
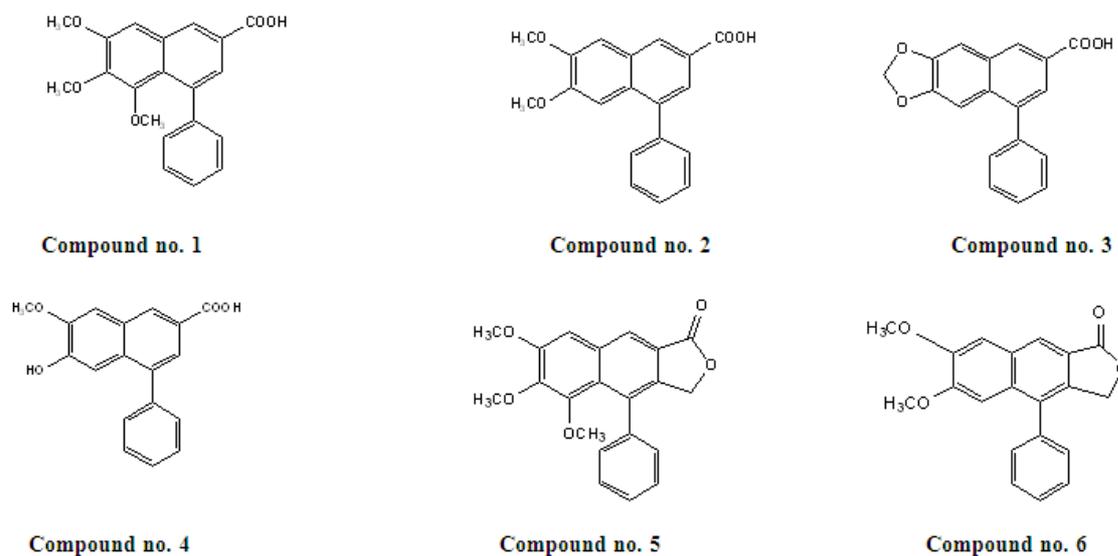
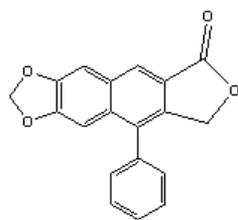
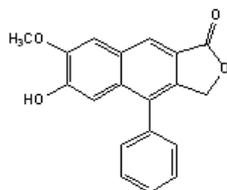


Figure 2: Effect of plant lignans on spleen cell proliferation

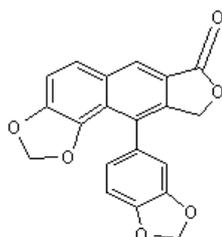




Compound no. 7



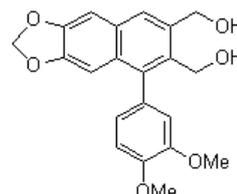
Compound no. 8



Helioxanthin

[*R. graveolens* extract (MeOH)]

Compound no. 9



Arylnaphthalene

[*J. gossypifolia* extract (Pet. ether)]

Compound no.10

Scheme-1 1-Phenylnaphthalene systems of synthetic and natural lignans

Table 2- Effect of *Ruta graveolens* and *Jatropha gossypifolia* on spleen cell proliferation

Sr. No.	Compound	100 µg/ml	200 µg/ml	400 µg/ml
1	MeOH extract of <i>Ruta graveolens</i>	141.896	191.103	262.827
2	Pet. ether extract of <i>Jatropha gossypifolia</i>	146.001	169.851	219.932
3	PHA (6 µg/ml)		617.26	
4	PBS		129.64	

All over findings suggest that both synthetic and naturally occurring 1-Phenylnaphthalene lignans positively modulates the immunity of the host although the activity at the doses used was less significant as compared to synthetic lignan compounds.

CONCLUSION

In summary, the naturally occurring and related synthetic derivatives of the Phenylnaphthalene lactone lignans possess significant immunomodulatory activity. In HIV treatment they will improve the function of the immune system of patients; can make the immune system competent in driving out the virus from the reservoirs. This appears to be an attractive and important addition to the present treatment of HIV which only offers antiretroviral drugs. Moreover, these compounds would be useful to understand the mechanism of diseases with higher levels of cellular activation, such as autoimmune and hyper sensibility diseases.

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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Abbreviations

CH ₂ N ₂	=	Nitrosomethylene
α	=	alpha
β	=	beta
γ	=	gamma
δ	=	delta
μ	=	micro
ml	=	milliliter
MeOH	=	methanol
Pet.ether	=	petroleum ether
PBS-T	=	Phosphate buffer saline –T
PHA	=	Phytohaemagglutinin
HCl	=	Hydrochloric acid
NH ₄ Cl	=	Ammonium chloride
CO ₂	=	Carbon dioxide
μl	=	micro litre
μg	=	microgram
ml	=	milliliter
ELISA	=	enzyme-linked immunosorbent assay