Indian Journal of Science and Technology, *Vol 12(5), DOI: 10.17485/ijst/2019/v12i5/141077, February 2019*
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Extraction and Transformative Reaction of Triterpenoid and Comparison of their Biological Activity

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Abstract

Objective: Fusion of heterocyclic ring system of the pentacyclic triterpenoids at position 2 and 3 of the steroidal nucleus has been found to afford chemically useful compounds. **Method/Analysis**: Lupeol (**I**) was used as the starting material to carry out a transformative reaction to introducing oxadiazole moiety to ring A of the triterpenoid which was identified as lupan (2,3-C) 1′,2′,5′-oxadiazole (**VI**). The structures of the compounds were established based on spectroscopic (UV, IR, NMR) analysis. **Finding:** The derivative of the pentacyclic triterpenoid obtained has been selected for their antibacterial, fungicidal and Phytotoxicity at different concentration with respect to parent compound and found oxadiazole derivative almost at all concentration give better result compare to parent compound. Despite their widespread existence in the vicinity and their direct and indirect defensive activities, systematic study regarding their biocidal activity has not been attempted sufficiently. Since limited chemical work has so far been attempted on pentacyclic triterpene skeleton so there is a possibility of ample opportunity to study the effect of number of reagents on them. **Novelty/Importance:** These investigations not only enrich the triterpene chemistry but at the same time may yield a number of very useful pharmacologically important derivatives.

Keywords: Biological Activity, Extraction, Phytotoxicity, Triterpenoids, Transformative Reaction

1. Introduction

Throughout history, humankind has always been interested in naturally occurring compounds from prebiotic, microbial, plants and animals sources. Various extracts of different parts of plants have been widely used in folk medicines and perfumes as well as in food flavor and preservatives and are more commonly utilized in chronic diseases like cancer, diabetes and asthma[1,2]. Plant secondary metabolite such as terpenoids is believed to be synthesized primarily as a chemical defense against harmful insects, bacteria and fungi and vertebrate herbivores[3]. Recently this group of compounds attracting attention from the pharmaceutical industry because of its considerable range of medicinal properties such as antibacterial, antifungal, anticancer and anti HIV activities[4,5]. The role of certain compounds is to act as chemical messengers, such as sex- attractants in insects, terrestrial and marine animals and humans. A number of derivatives of them have also been prepared either to establish the structure of the compound or to study the reactivity of such compounds towards some specific oxidizing and reducing agents[6–8].The author interested in the biocidal activity of these plant products include understanding their mode of action, determining Structure Activity Relationship (SAR) and making suitable derivatives with improved biocidal activity.

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2. Materials and Method

2.1 Isolation of the Compound from the Barks of *Xanthoxylum budrunga*

The powdered plant materials were collected from the Sukna belt of Darjeeling foothills (barks of *Xanthoxylum budrunga*) and extracted with toluene using soxhlet apparatus for 36 hours. The solvents were then removed under reduced pressure and a sticky brown residue was obtained. This residue of the compounds present in the bark of the plant was than purified by column chromatography[9] using silica gel (60-120) mesh and suitable proportions of petroleum ether and ethyl acetate mixture as the eluent.

2.2 Elucidation of the Structures

All the melting points were determined by open capillary method and are uncorrected. The NMR spectra were recorded in $CDCl_3$ solutions at ambient temperature on a Bruker Avance 300 MHz-FT NMR spectrometer using 5mm BBO probe. The chemical shift δ are given in ppm related to Tetra Methyl Silane (TMS) as internal standard. The coupling constant (*J*) are reported in Hz. The IR spectra were recorded in Shimadzu FT-IR spectrophotometer in KBr discs.

2.3 Characterization of the Isolated Compounds

Different fractions of isolated compounds were mixed and crystallised by chloroform and methanol mixture which gave white crystals, m.p. 210– 212°C; $[\alpha]_D$= +30.4 (conc. 0.58 in $CHCl_3$). Its IR spectrum exhibited hydroxyl at ν_{max}3610, 1020 cm^{-1} and exomethylene at ν_{max}3070, 1640, 887 cm^{-1} absorption and was identified as lupeol (**I**)[10] from other spectral data (NMR, Mass) and by comparison with authentic sample of lupeol (**I**) (Figure 1).

2.4 Hydrogenation of Lupeol: Preparation of Lupanol(II)

Lupeol (**I**) dissolved in a mixture of ethyl acetate and acetic acid (100 ml each) was shaken in an atmospheric hydrogen in presence of PtO_2catalyst. The solid obtained after crystallization by using a mixture of chloroform and methanol had m.p. 204°C, $[\alpha]_D$= −15°. IR spectrum of the compound showed peak at 3330 cm^{-1} for hydroxy functional group. This compound was found to be identical with an authentic sample oflupanol(**II**) (mmp, CO TLC, CO IR) [Lit[11]m.p. 206°C, $[\alpha]_D$= -17.8°] (Figure 2).

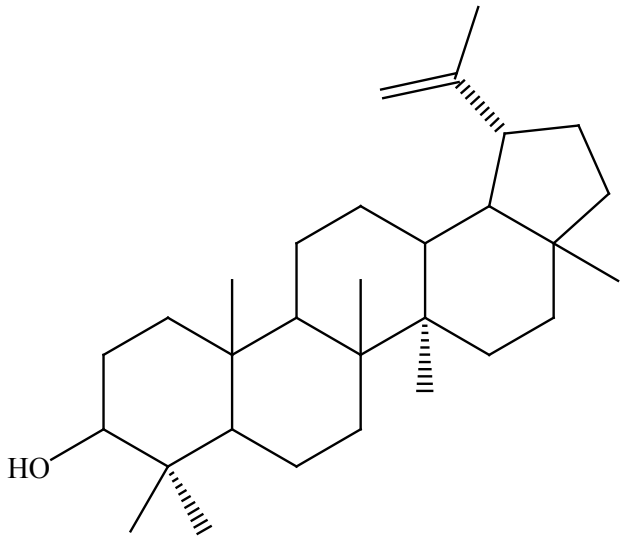

Figure 1. Lupeol (I).

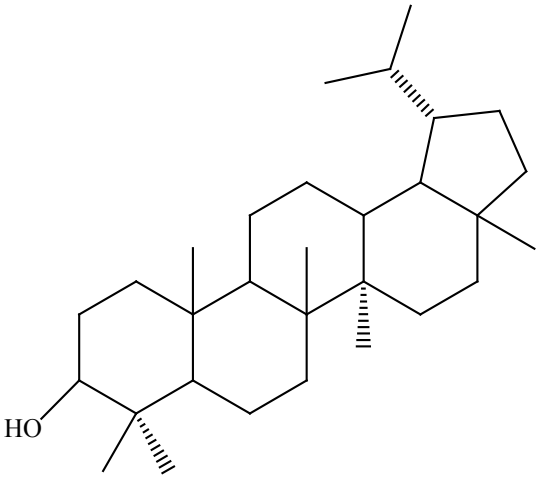

Figure 2. Lupanol (II).

2.5 Jone's Oxidation of Lupanol(II): Preparation of Lupanone(III)

To a solution of lupanol(**II**) in pure acetone, Jone's reagent was added until complete the reaction. The resulted mixture dissolved in benzene and chromatographed over a column of silica gel developed with petroleum ether. The chromatogram on elution with pet. ether: ethyl acetate (70:30) furnished different fractions of a single compound. Different fractions were mixed and crystallized by using chloroform and methanol mixture. The crystallization furnished colorless solid, m.p. 208-9°C, $[\alpha]_D$= + 15°, IR

1710 cm^{-1} for carbonyl group. It did not respond to the Beilstein test for halogen and to the TNM test for unsaturation. The compound was found to be identical (mmp, CO IR, CO-TLC) with an authentic sample of lupanone(**III**) [Lit[10]m.p. 210-11°C, $[\alpha]_D$= +16.2°] (Figure 3).

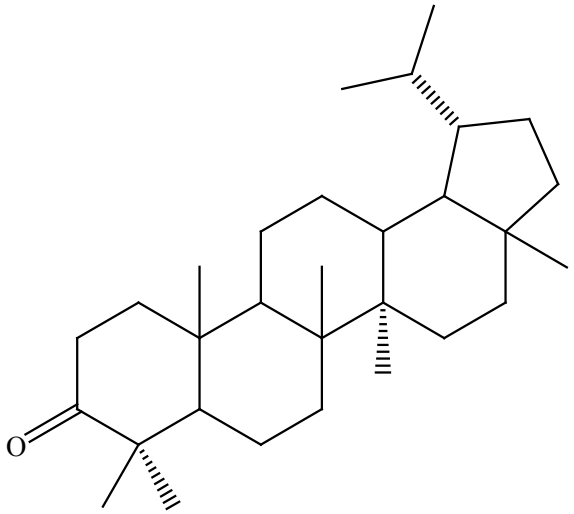

Figure 3. Lupanone (III).

2.6 Preparation of 2, 2-Dibromolupanone (IV) and 2α-Bromolupanone (IVa)

A solution of lupanone (**III**) was mixed with dimethylsulphoxide. N-bromo succinimide was added. The resulted mixture was chromatographed over a column of silica gel. The chromatogram on elution with petroleum ether only furnished a single compound 2, 2-dibromolupanone (**IV**) and further elution with pet. ether: ethyl acetate (80: 20) furnished another single compound 2α-bromolupanone(**IVa**) (Figure 4) and (Figure 5).

2.7 Preparation of 2, 3-Dioximino Lupane (V)

2, 2-Dibromolupanone (**IV**) dissolved in pyridine was refluxed with hydroxyl amine hydrochloride in ethanol. The compound obtained from the reaction was purified by repeated crystallization from chloroform-methanol mixture to obtain a white amorphous powder of the compound analyzed for $C_{30}H_{50}O_2N_2$, m.p. 193°C, $[\alpha]_D$= +21.6°. IR spectrum showed peaks at 3200-3400 cm^{-1} (C=N). It exhibited UV absorption maximum at 220 nm (€=5100). Mass spectrum of the compound showed moleculer ion peak at m/z 469 $[M]^+$ the other peaks at prominence appearance at m/z 441, 439, 425, 424(base peak), 422, 380, 341, 340, 299, 231, 191, 163, 149, 136, 122, 121, 95, 81, 69. PMR spectrum of the compoundcould not be taken owing to solubility problem. Thus on the basis of the above spectral data the compound was identified as 2, 3-dioximino lupane(**V**) (Figure 6).

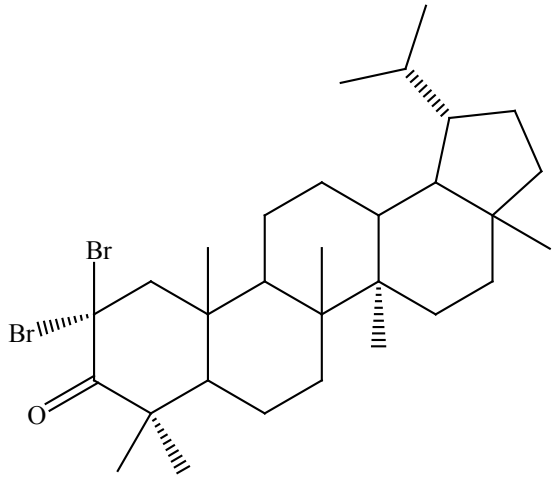

Figure 4. 2, 2-Dibromolupanone (IV).

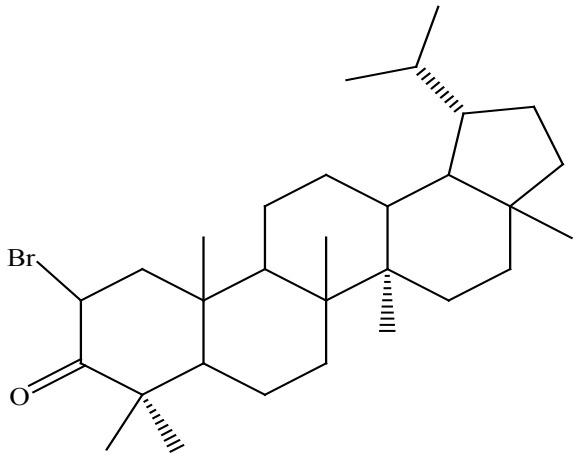

Figure 5. 2 α-Bromolupanone (IVa).

2.8 Preparation of Lupan[2,3-C] $1^{/},2^{/},5^{/}$-oxadiazole (VI)

Lupan [2, 3-C] $1^{/},2^{/},5^{/}$-oxadiazole(**VI**) was prepared by cyclization of 2, 3-dioximino lupine (**V**) in dry DMF under microwave irradiation (100W, 100°C) for 10 minutes. The compound obtained from the reaction was purified by repeated crystallization from chloroform-methanol mixture to obtain a compound (**VI**), m.p.249-50°C, analyzed for $C_{30}H_{48}ON_2$, which was corroborated by mass spectral analysis that showed the molecular ion at m/z 452. The other ions appeared at m/z 437 $[M\text{-}CH_3]^+$, 409 [M-CH

$(CH_3)_2]^+$, 367,271, 259, 245, 231, 206, 191, 163, 149, 123, 121, 109, 95, 81, 55. IR spectrum of the compound showed peaks at 1620 cm^{-1} (—C=N-O) and 890 cm^{-1} for heterocyclic ring. It showed UV absorption maximum at 223 nm (€=5169) for disubstituted furazan derivative[11]. Interestingly in the NMR spectrum different methyl group signals got separated which may be due to the presence of heterocyclic ring attached with ring[12]. It showed the presence of six tertiary methyl of which four of them have been shifted downfield about 0.5 ppm in comparison to that oflupanone (**III**). A pair of one-proton doublets each at 2.1 (J=16 Hz) ppm and 3.15 (J= 16 Hz) ppm may be due to the germinal coupling of the C-1 proton which are adjacent to the furazan ring. Thus from spectral analysis the structure of the compound has been established as lupan[2,3-C]1′,2′,5′-oxadiazole(**VI**) (Figure 7).

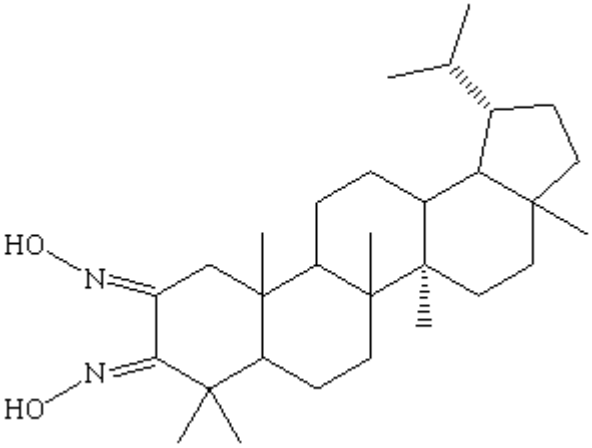

Figure 6. 2, 3-Dioximino lupane (V).

2.9 Assay of Antibacterial Activity by the Disc Diffusion Method

One ml of 48 h old culture of the test bacterium was taken in a Petri dish of 90 mm diameter. Then 20 ml of sterile Nutrient Agar (NA) medium was poured in the Petri dish and shaken carefully to mix the bacterial suspension with the medium. The Petri dishes were allowed to cool. Filter paper discs of 5 mm diameter containing desired concentration of the test samples were placed on the surface of the solidified media and incubated at 37°C in an incubator for 48 h. Diameter of the inhibition zones were measured. In control sets, no chemicals were used in the filter paper discs but sterile distilled water was used to soak the filter papers.

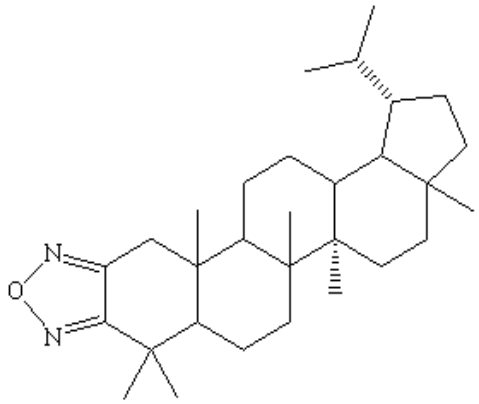

Figure 7. Lupan [2,3-C]1′,2′,5′-oxadiazole (VI).

2.10 Assay of Antifungal Activity

Fungi were grown on Potato Dextrose Agar (PDA) medium at 28±1°C for mycelial growth. The fungicidal activities were determined using agar cup bioassay and spore germination bioassay. The purified eluents (10ml) were placed on two spots 3 cm apart on a clean grease free slide and the solvent was allowed to keep for some time to evaporate. One drop (0.02 ml) of spore suspension (10spores/ml) prepared from 15 days old culture of the test fungi was added on the same place where the purified eluents were placed and subsequently evaporated. Various compounds of five different concentrations were prepared (500 ppm, 400 ppm, 300ppm, 200 ppm, 100 ppm). The studies were performed at 28±1°C for 24 h under humid conditions in Petri plates. Finally after proper incubation period, one drop of a cotton blue-lacto phenol mixture was added to each spot to fix the germinated spores.

The number of spores germinated was compared with that of germinated spores of control (where no chemicals were used). Moist chamber was used for germination of spores. The number of germinated spores was calculated on the basis of an average of 300 spores per treatment.

2.11 Phytotoxicity

In order to show phytotoxic activities different concentrations of compounds were prepared and applied to check germination of both root and shoot of the germinating seeds. Phytotoxicities of the parent compound and its derivative were determined on the healthy seeds of wheat (Sonalika variety), rice (IR-20, Jaya variety) and pea purchased from the local market. These healthy seeds were dipped in acetone-water suspensions of the compounds

of different concentration(500 ppm, 250 ppm, 100 ppm) and incubated for 1, 4 and 8 hours. The treated seeds of wheat, rice and pea were allowed to germinate on a mat of moist filter paper. The roots and shoots of germinated seeds were kept in a covered Petri plates. Each experiment was based on 80 seeds of each varieties or plants. After five days the germinated seeds (treated with compounds) were counted. Treated experimental sets were compared with that of control sets where no compounds were used to treat the seeds. Each experiment was repeated in triplicate. All apparatus and materials used were sterilized where necessary.

3. Result and Discussion

3.1 Antibacterial Activity

Two different compounds oxadiazole derivativelupan [2,3-C]1′,2′,5′-oxadiazole (**VI**) and (Table 1) parent compound lupeol(**I**) were tested for their antibacterial properties against five different bacteria *E. coli, B. subtilis, S. aureus, Lactobacillus* and *Pseudomonas*.

It was evident that significant inhibition of growth was observed in the case of *E. coli* and *Lactobacillus* by all the concentrations ofoxadiazole derivative (**VI**)with respect to lupeol(**I**)tested in disc diffusion method. For oxadiazole derivative (**VI**) maximum inhibition was observed at 500 ppm when tested on *E. coli*; the inhibition zone was 2.9 cm, but at 250 ppm and 100 ppm concentration the diameter of inhibition zones were 2.5 cm and 2.6 cm respectively. Thus there is a gradual increase in the zone of inhibition with the increase in concentration of the compounds. Similar results were also observed in case of the parent compound (**I**) at 500 ppm, 250 ppm and 100 ppm concentrations the diameters of inhibition zones were 2.7 cm, 2.5 cm and 2.4 cm respectively.

Although the inhibition was found relatively less with *B. subtilis, S. aureus* in comparison to above two microorganisms but the trends of activity were found identical.

The growth of inhibition zone with *Pseudomonas* was not prominent for both (**VI**) and (**I**).

3.2 Antifungal Activity

The antifungal activities of lupeol (**I**) and lupan [2,3-C]1′,2′,5′-oxadiazole (**VI**) at different concentrations were tested against (Table 2).

The parent compound (**I**) and the respective oxadiazole derivative (**VI**) showed inhibition of spore

Table 1. Results of antibacterial activity

Microorganism	Compounds under investigation	Inhibition zone (cm)		
		Concentration		
		100 ppm	250 ppm	500 ppm
E. coli	**VI/I**	2.5 / 2.4	2.6/2.5	2.9/2.7
B. subtilis	**VI/I**	1.7/1.4	1.8/1.7	2.1/2.1
S. aureus	**VI/I**	1.6/1.5	1.7/1.6	1.8/1.7
Lactobacillus	**VI/I**	2.3/1.7	2.4/1.9	2.6/2.2
Pseudomonas	**VI/I**	NP/NP	0.8/NP	1.0/NP

NP = Not prominent, I=Lupeol, VI= lupan [2,3-C]1′,2′,5′-oxadiazole

Table 2. Results of antifungal activity

Compounds	Concentration	% of germination	% of inhibition	Range of Germ tube length
VI/I	Control	96.77	0	3.0-5.0
	100 ppm	12.30 /83.88	86.64 /16.04	2.4-5.4 / 2.4-4.8
	200 ppm	17.89 /8.04	81.56 /91.27	2.2-4.8 / 2.0-4.4
	300 ppm	16.19 /5.16	83.31 /94.40	2.2-4.4 / 2.0-4.2
	400 ppm	9.52 /2.48	91.19 /97.31	2.2-4.0 / 1.6-3.6
	500 ppm	4.43 /1.02	95.64 / 98.90	2.2-3.4 / 1.6-3.0

I=Lupeol, VI= lupan [2,3-C]1′,2′,5′-oxadiazole

Table 3. Results of phytotoxicity

Compound	Seeds of plants	Concentration	Germination of seeds	
			Length of Root (cm)	Length of Shoot(cm)
I/VI	Rice	Control	GM, 0. 68	GM, 0.30
		100 ppm	GM, 0.59 / 0.60	GM, 0.24 / 0.25
		250 ppm	GM, 0.52 / 0.55	GM, 0.21 / 0.20
		500 ppm	GM, 0.43 /0.52	GM, 0.17 /0.10
	Wheat	Control	GM, 2.62	GM, 1.1
		100 ppm	GM, 2.52 / 2.50	GM, 0.77 / 0.91
		250 ppm	GM, 2.4 / 2.12	GM, 0.65 / 0.75
		500 ppm	GM, 2.10 /1.18	GM, 0.45 / 0.43
	Pea	Control	GM, 2.6 / 1.63	GM, 1.16 / 1.16
		100 ppm	GM, 1.65 / 0.65	GM, 0.74 / 0.52
		250 ppm	GM, 1.36 / 0.39	GM, 0.56 / 0.33
		500 ppm	GM, 1.00 / 0. 30	GM, 0.45 / 0.22

GM= Germinated, I=Lupeol, VI= lupan [2,3-C]1′,2′,5′-oxadiazole

germination and inhibitory effect on the growth of *F. solani*. Parent compound (**I**) at 100 ppm concentration showed 83.88% germination where as 96.77% germination was experienced in control set. Similar results were also observed with oxadiazole derivative (**VI**) at the same concentrations. However, at 500 ppm concentration lupeol (**I**) significantly reduced germination of spores. It showed 98.90% inhibition of spore germination.

Oxadiazole derivative (**VI**) showed 12.30% germination at100 ppm concentration. The 500 ppm concentration of oxadiazole derivative (**VI**) significantly reduced germination of spores. It showed 95.64% inhibition of spore germination.

3.3 Phytotoxicity

The phytotoxic effects of parent compound lupeol(**I**) and oxadiazole derivative(**VI**) on the germination of *Triticum aestirium* (wheat), *Oryza sativa* (rice) and *Pisum sativum* (pea) seeds have been tested (Table 3).

In case of rice, the parent compound lupeol(**I**) at 100 ppm concentration showed 0.59 cm root germination and 0.24 cm shoot germination; whereas 0.68 cm root and 0.30 cm shoot germination was observed in the case of control set. Higher concentrations of the compounds gradually reduce the germination of root as well as shoot.

In case of wheat, the parent compound (**I**) at 100 ppm concentration showed 2.52 cm root germination and 0.77 cm shoot germination, in comparison to 2.62 cm root and 1.1 cm shoot germination experienced in control set. The rate of germinations (root and shoot) was found to be reduced at higher concentrations of the compounds.

In case of pea, the parent compound (**I**) at 100 ppm concentration showed 1.65 cm root germination and 0.74 cm shoot germination, in comparison to 2.6 cm root and 1.16 cm shoot germination observed in control set. The rates of germinations (root and shoot) were found to be reduced at higher concentrations of the compounds.

Similar results were observed when the experiments were carried out with the respective oxadiazole derivative (**VI**). In case of rice, at 100 ppm concentration showed 0.60 cm root germination and 0.25 cm shoot germination, whereas 0.68 cm root and 0.30 cm shoot germination was experienced in control set. At 500 ppm concentration slightly reduced the germination of both root and shoot. It showed 0.52 cm root germination and 0.10 cm shoot germination.

In case of wheat, the oxadiazole derivative (**VI**) at 100 ppm concentration showed 2.5 cm root germination and 0.91 cm shoot germination; whereas 2.62 cm root and 1.1 cm shoot germination was observed in control set. At 500 ppm concentration slightly reduced the germination of both root and shoot. It showed 1.18 cm root germination and 0.43 cm shoot germination.

In case of pea, the oxadiazole derivative (**VI**) at 100 ppm concentration showed 0.65 cm root germination and 0.52 cm shoot germination; whereas 1.63 cm root and 1.16 cm shoot germination was observed in control set. The 500 ppm concentration slightly reduced the germination of both root and shoot. It showed 0.30 cm root germination and 0.22 cm shoot germination.

4. Conclusion

The derivative showed better activity than parent compound. The introduction of oxadiazole moiety to ring A of the pentacyclic triterpenoid enhanced the activity of the compound obtained from natural sources. The present study will be extremely helpful to enrich the present knowledge about the structure activity relationship for this type of triterpenoid skeleton and encouraged the author towards a biological potential on a library of pentacyclic triterpenoids and their derivatives.

5. Acknowledgement

The author expresses deep sense of gratitude to Supervisor Prof. Pranab Ghosh, Department of Chemistry, University of North Bengal and Authorities of Raiganj University for providing Laboratory facilities and support.

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