

# Analgesic and Toxicologic Analyses of Purple Rain (*Dendrobium superbum*)

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**ABSTRACT:** This study primarily aimed to perform phytochemical screening and pharmacological testing on a locally abundant orchids that grow widely in Cervantes, Ilocos Sur known as purple rain (*Dendrobium superbum* Reichb.) This experimental investigation determined the chemical substances present in both the stem and leaves and determine the pharmacological effects of the ethanol extract using male Swiss mice as test animals in terms of analgesia and toxicity.

The following chemical substances like alkaloids, glycosides, tannins, saponins and sterols were found to be present in both the stems and leaves of purple rain (*Dendrobium superbum* Reichb.).

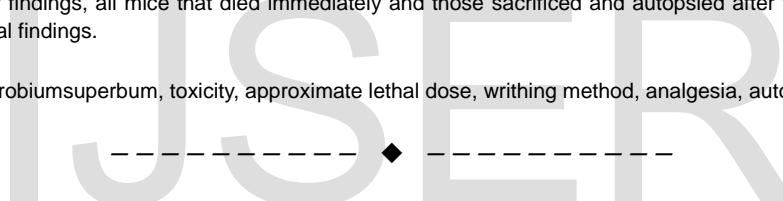
There are pharmacological effects of the ethanol extract (screened) substances using male Swiss mice as test animals.

Under the conditions of this test, the ethanol extract showed that it has an analgesic activity when administered orally to male Swiss mice; it produced a 6.25%, 31.25% and 56.25% protection against writhing at 500, 1000 and 1,500 mg/kg, respectively.

The approximate Lethal Dose (ALD) of the sample ethanol extract of purple rain administered orally in male Swiss mice is 2,500mg/kg. Toxidrome ranged from ptosis, decreased motor activity and convulsion leading to the deaths of male Swiss mice.

In the autopsy findings, all mice that died immediately and those sacrificed and autopsied after fourteen (14) days had grossly normal findings.

**Keywords:** *Dendrobium superbum*, toxicity, approximate lethal dose, writhing method, analgesia, autopsy



## 1.1. INTRODUCTION

The municipality of Cervantes, Ilocos Sur, Philippines is very fortunate because of the abundance of the so-called aristocrat of the plant world, the "orchid." This plant excites wonder because of its complex structure. Cervantes is long remembered because of this and because it has a suitable climate for the growth of orchids.

The municipality of Cervantes is situated within the Cordillera Mountains lying at the southeastern tip of the province of the Ilocos Sur. It is bounded by Tadian, Mt. Province of the east, Mankayan and Bakun, Benguet to the south; Alilem, Ilocos Sur on the Southwest; Sigay, Ilocos Sur on the West; Gregorio del Pilar, Ilocos Sur on the northwest; and Quirino, Ilocos Sur (Angaki) on the north.

Cervantes is 399 kilometers northeast of Metro Manila, 169 kilometers east of Vigan, the Capital town of the Province, 123 kilometers north of Baguio City, the summer Capital of the Philippines, and 19 kilometers north of Mankayan, Benguet, the northeast minor urban center of the Philippines.

Due to location and natural barrier, the town is almost completely isolated from the rest of the Ilocos Sur. Poverty deprives some of the residents (especially those in far-flung areas) of

their basic needs. Deprivation makes them Susceptible to diseases. It is imperative, therefore that easy, reliable and cheap means of curing illness be made available to the masses.

It is time, therefore, to give medicinal plants just scientific treatment not only as legitimate area of scientific inquiry but also as an issue for national concern. We can think of reasons why:

1. Plant preparations are the only medicines many of our rural folks have easy access to notwithstanding miniature pills and bottled drugs.
2. There is a need to develop local sources of drugs in the name of saving dollars and self-reliant economy.
3. Medicinal plants have their place in modern medicine.

With the reason stated above, the researcher was greatly interested to look into the medicinal values of indigenous “sanggumay” orchid (purple rain) that grows abundantly in Cervantes, Ilocos Sur. If found to have medicinal properties, it will attract scientists to discover the application of its therapeutic properties thus it will be a source of remedial agents for the treatment of quite a number of ailments. *Dendrobiumsuperbum Reich.* is not yet included in the list of medicinal plants, so if found to have medicinal properties then it may also be added to the compilation of data and documentation of Philippine Medicinal Plants.

This study primarily aimed to perform phytochemical screening and pharmacological testing of purple rain plant.

It determined some chemical substances which have medicinal properties present in the stems and leaves of purple rain (*DendrobiumsuperbumReichb.*)

It also determined the pharmacological effects of the screened substances on the male Swiss mice as test animals in terms of analgesic and toxicity effects.

This study was limited only to the phytochemical screening and pharmacological testing of “sanggumay” orchid.

Only the stems and leaves were used in the experimental investigation. The determination of the chemical constituents was limited to the qualitative rather than the quantitative analysis.

In the pharmacological testing, it was limited the analgesic and toxicity effects of the test drug from the stems and leaves of purple rain using male Swiss mice as test animals.

## 1.2.FRAMEWORK

One of the orchids species with a powerful fragrance is *Dendrobiumsuperbum*. This species is native to New Guinea, the Philippines and north to Sri Lanka and Thailand. The long slender pseudobulbs are said to grow to 3 meters (nearly 10 feet) in length. However, in

cultivation, especially when it is mounted, the pseudobulbs often do not even reach three feet. When well grown in a pot, the pseudobulbs can easily reach five to six feet. This species has lavender flowers that have two burgundy spots on either side of the lip towards the inner base. The flowers of some forms reach only half-open, while others open fully. Although the literature says that each node produces 1-3 flowers, my specimen-size plants produced four flowers on many nodes. Although I enjoy it, the fragrance of these flowers can be overpowering to some people. It smells like raspberry, strawberry, rhubarb, or hyacinth, depending upon one's imagination. Even the dried up flowers keep the fragrance for a while (Wang, 2015).

Some international studies were used as guide in this research:

Senguttuvan et al.2014 in their study , “Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaerisradicata* L. for *in vitro* antioxidant activities” mentioned that phytochemical analysis made for the leaf and root parts of *H. radicata* revealed the presence of alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids. These secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses. The extraction yield calculated for petroleum ether, chloroform, ethyl acetate, methanol and water extracts of both parts of *H. radicata* showed that methanol extract registered higher percentage of yield. It may be due to high polarity of methanolic solvent which can draw high variety of plant constituents than the other solvents did.

Yusuf, A . et al.(2014) in their study, Phytochemical analysis of the methanol leaves extract of *Paulliniapinnatalinn* mentioned that The phytochemical constituents of the leaf of *P. pinnata* was investigated. The leaf was found to constitutesteroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids. The leaf is an African woody vine widely used in traditional medicine for the treatment of malaria and as a remedy against different forms of pains and as a natural cure (Jimoh et al., 2007). The presence of the constituents was also found to be similar to those reported for most medicinal plants. In East Africa, the leaves are used against snake bites, rabies, mental problems, blindness and eye troubles, together with the roots, against gonorrhoea, paralysis, wounds, threatened abortion, malaria, ancylostomiasis, and to expel the placenta. Roots are applied against eczema, as a tonic and as a styptic medicine (Abourashed et al., 1999). The whole plant is applied for bad skin conditions, for wounds and microbial infections. The root decoction is drunk in the case of nausea and vomiting. In Nigeria, the research work is still in the initiation stage and the work was carried out to find its phytochemical constituents in relation to the leaf elsewhere in Africa.

Veeruraj, Anguchamy et al. (2016), in their study ,Isolation and Identification of Cytotoxic and Biological Active Toxin from the Puffer Fish *Arothronstellatus* investigated the biological, biochemical and cytotoxic effects of puffer fish (*Arothronstellatus*) toxin extracts under in-vitro condition. Extracted toxins from various organs of puffer fish were purified by using active charcoal column, and Bio-gel-P2 column chromatography. The lethality of toxin was tested in crabs, which consists of neurotoxic compounds. The degree of the brine shrimp lethality assay was found directly proportional to the concentration of the toxin extracts, which

was well supported by hemolytic assay. The experimental results suggested that the gonad was found higher toxins than the liver and muscles. The mortality rate of brine shrimp nauplii was increased with the raise of concentrations of toxin level. Among the different doses and time dependent cytotoxic effect of human cervical carcinoma (HeLa) cells were showed  $4.0 \mu\text{g}/\text{mL}$  of toxin, which was effectively inhibited cancer cell proliferation. HPLC and TLC analysis was revealed that the *A. stellatus* toxin contains tetrodotoxin (TTX), related compounds 4-epi TTX and anhydro-TTX. The results suggested that the *A. stellatus* contain TTX as a major and anhydro-TTX as a minor toxin. Further they revealed that it could be the potential candidate in the field of anticancer drug discovery against human cervical cancer cells. Their data is confirmed that the puffer fish toxin as an interesting source of novel bioactive natural compounds with potent applications in pharmacology.

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### 1.3 RESEARCH DESIGN

The figure presents the research paradigm.

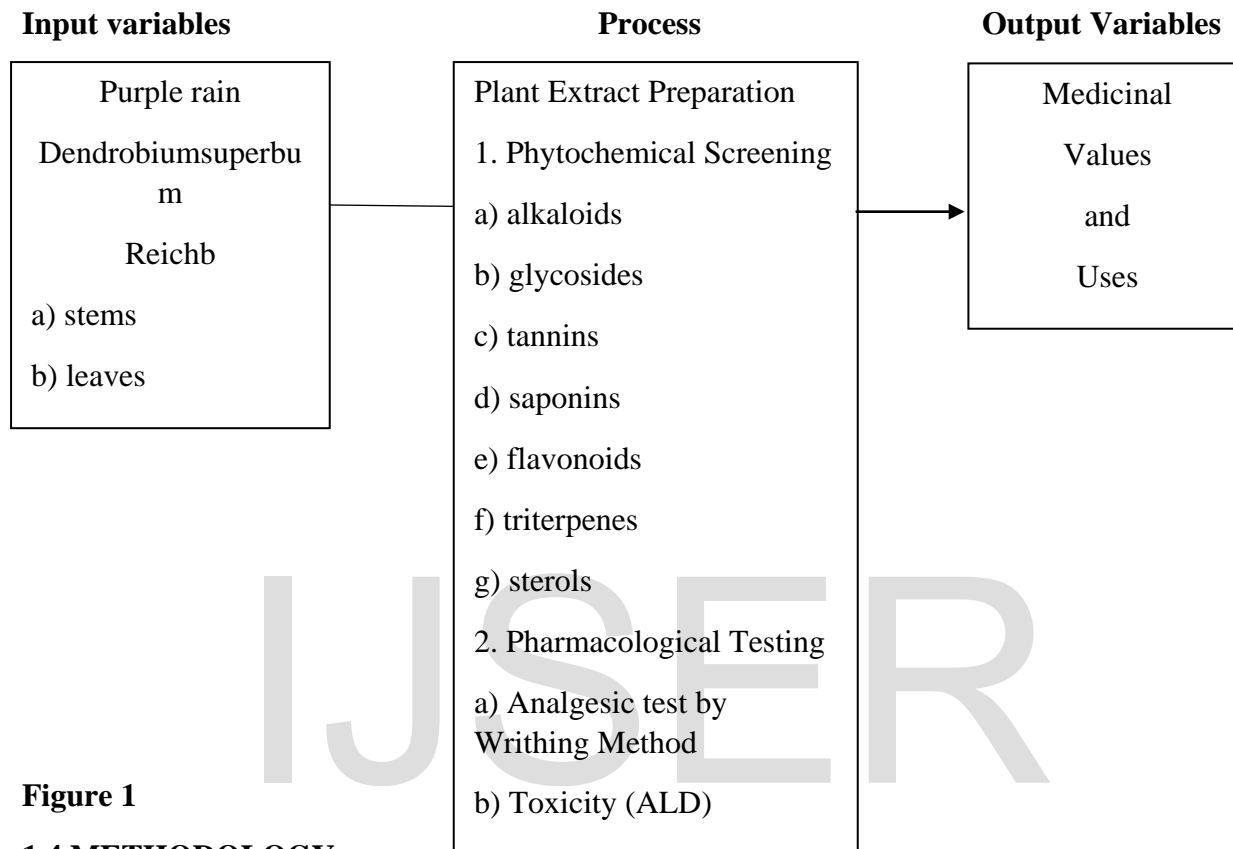


Figure 1

### 1.4 METHODOLOGY

#### Design of the Study

This study made use of the experimental research design in actual laboratory set-up.

*Phase I.* The gathering of fresh stems and leaves of purple rain; the air drying process of stems and leaves for a month and the extraction process (ethyl alcohol) were included in the phase.

*Phase II.* This phase included the phytochemical screening of the stems and leaves of purple rain.

The following tests were used:

Mayer's Test for the determination of the presence/absence of alkaloids.

Fehling's Test for the determination of the presence/absence of glycosides.

Test for the determination of the presence/absence of Tannins.

Froth Test for the determination of the presence/absence of Saponins.

Color Test for the determination of the presence/absence of Flavonoids.

Liebermann-Burchard Test for triterpenes and sterols.

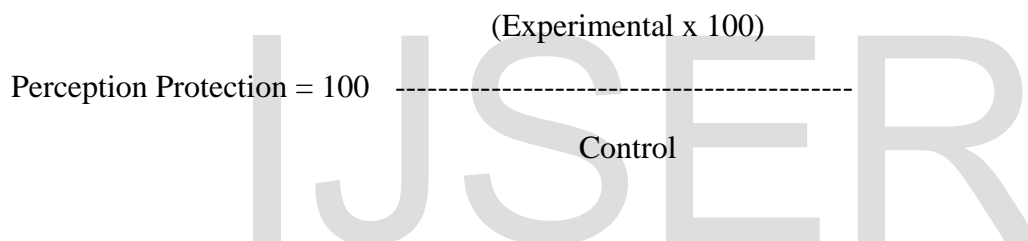
*Phase III.* This included the pharmacological testing.

#### *Experimenting Animals*

Healthy Swiss mice each weighing 17 to 30g at the start of the experiment were kept in individual observation cages. All animals were fasted from food and water 16 hours before the test. Two hours after administration of the drug, the animals were given free access to food and water.

#### *Analgesic Test by Writhing Method (PBO)*

Three increasing dose of the test material were given orally to the animals in groups of five including the negative (normal Saline solution) and positive (aspirin) controls. Thirty minutes after dosing, the mice were injected intraperitoneally with phenylbenzoquinone (PBO). The number of writhing and number of animals without writhing were closely observed.



#### *Approximate Lethal Dose (ALD)*

Seven increasing dose of the test substance were given orally to male Swiss mice in groups of two including the control to determine the dose that causes death to experimental animals.

### **1.5 RESULTS AND DISCUSSION**

**Table 1.**Phytochemical Screening of purple rain

Substance Determined	Method Used	Results	
		Stems	Leaves
1. Alkaloids	Mayer's Test	+	+
2. Glycosides	Fehling's Test	+	+
3. Tannins	Gelatin Test	+	+
4. Saponins	Froth Test	+	+
5. Flavonoids	Color test	-	-
6. Triterpenes	Liebermann-Burchard Test	-	-
7. Sterols	Liebermann-Burchard Test	+	+

Legend: + = presence of substance determined  
- = absence of substance determined

### INTERPRETATION OF FINDINGS

Findings of this study show that the plant contains medicinal properties like alkaloids, glycosides, tannins, saponins and sterols. The presence of these therapeutic substance confirmed the purported use of purple rain as medicinal plant.

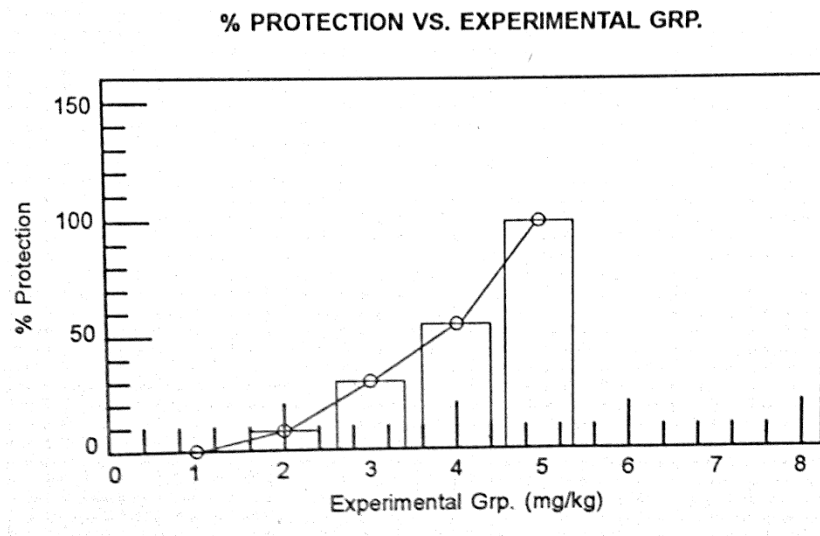
### Pharmacological Screening

**Table 2. Results of Analgesic Test by Writhing Method**

Group No.	Drug	Dose mg/kg	N	No. of Mice w/o Writhing/ Total No. of Test Animals	No. of Writhing	Percent Protection
I	Control	0	4	0/4	16	-
II	Sanggumay	500	4	3/4	15	6.25
III	Sanggumay	1000	4	2/4	11	31.25
IV	Sanggumay	1500	4	2/4	7	56.25
V	Aspirin	200	4	4/4	0	100.00

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Percent Protection =  $100 - [(Experimental \times 100)/control]$



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**Figure 2**  
**Group Showing the results of Analgesic Test**  
**By Writhing Method (PBO) Using Male**  
**Swiss Mice as Test Animals**

**INTERPRETATION OF FINDINGS**

Under the condition of this test, the ethanol extract of purple rain showed that it has an analgesic activity when administered orally to male Swiss mice, it produced a 6.25%, 31.25% and 56.25% protection against writhing at 500, 1000 and 1,500 mg/kg, respectively. However, the dose of 500 mg/kg did not produce significant protection.

The ethanol extract dose (mg/kg) of the experimental group is directly proportional to the present protection of the male mice. (See Figure 2) This showed that as the dosage increased, the greater the percent protection of the male Swiss mice.

As long, as it is below the toxidrome which is 2,500 mg/kg, the higher the dosage injected intraperitoneally to male Swiss mice, the more is the percent protection against writhing.

The positive control (aspirin) showed the highest percent protection.

**Table 3. Results of Approximate Lethal Dose (ALD) Using Male Swiss Mice as Test Animals**

Group No.	Dose mg/kg	n	No. of Mice with Positive Signs (Deaths)/Total No. of Mice Tested				
			Day 1	Day 2	Day 3	Day 4	...Day 14
1	0	2	0/2	0/2	0/2	0/2	0/2
2	1,000	2	0/2	0/2	0/2	0/2	0/2
3	2,000	2	0/2	0/2	0/2	0/2	0/2
4	2,500	2	2/2	-	-	-	-
5	3,000	2	2/2	-	-	-	-
6	3,500	2	2/2	-	-	-	-
7	4,000	2	2/2	-	-	-	-
8	8,000	2	2/2	-	-	-	-

n = number of mice tested

**Table 4. Behavioral Observation/Toxidrome of oral Administration of the Ethanol Extract of “Sanggumay” Orchid in Male Swiss Mice**

Dose Mg/kg	n	Observation
0	2	No effect
1,000 2,000	2	Five (5) minutes after dosing, the mice manifested ptosis and decreased motor activity which lasted for one (1) hour and fourteen (14) minutes. No death occurred within the fourteen (14) days period.
2,500	2	Four (4) minutes after dosing, the mice manifested ptosis and decreased motor activity and convulsion leading to the death of mice. Two (2) mice died within two (2) hours and thirty (30) minutes after dosing.
3,000 3,500 4,000 8,000		Four (4) minutes after dosing, the mice manifested ptosis and decreased motor activity and convulsion leading to the death of all mice. All mice died within one (1) hour and twenty (20) minutes after dosing.

The approximate Lethal Dose (ALD) of the ethanol extract of “Sanggumay” orchid administered orally in male Swiss mice is 2,500 mg/kg. Toxidrome ranged from ptosis, decreased motor activity and convulsion leading to the deaths of mice.

### Autopsy Findings

All mice that died immediately and those sacrificed and autopsied after fourteen (14) days had grossly normal findings.

### 1.6 CONCLUSIONS

1. The ethanol extracts of the stems and leaves of purple rain (DendrobiumsuperbumReichb.) contain therapeutic substances such as alkaloids, glycosides, tannins, saponins and sterol. This implies that the plant is a good source for the treatment of hypertension, tumor, congestive heart failure, wounds, sores, boils, stomach ache, diarrhea, sore throat, burns and ulcer, nasal congestion, hemorrhage, malaria and other rectal disorders.

2. The ethanol extract of purple rain orchid has analgesic effect when administered orally to male Swiss mice.

The ethanol extract of purple rain has toxicity effect when administered orally to male Swiss mice. The Approximate Lethal Dose is 2,500 mg/kg.

## 1.7 RECOMMENDATIONS

1. A follow-up study should be conducted to quantify, isolate and identify the type of alkaloids, glycosides, tannins, saponins and sterols present in the stem and leaves.
2. The roots and flowers of the plant are recommended for phytochemical screening and pharmacological testing.
3. The roots, stems, leaves, and flowers of the plant are recommended for microbiological assay.
4. Further studies on the plant's therapeutic properties are recommended to be undertaken by interested drug companies.
5. Purple rain is recommended to be listed in the compilation and documentation of Medicinal Plants in the Philippines through the National Research Council of the Philippines, Department of Science and Technology and the University of the Philippines.

## 1.8 REFERENCES

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