Extract of Lyonia ovalifolia (Wall.) Drude as a Potent Rodenticide

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Abstract

Phytochemicals are naturally synthesized in all parts of the plant body. Lyonia ovaliafolia (Wallich) has been used in a traditional medicine for the treatment of skin diseases such as scabies and itching by different local communities of Nepal. The study was done to analyze the active phytochemicals present in alcoholic extract of its leaves. The sample was random collection along the road, from Namobuddha. A range of chemical tests were adopted for analyzing the types of phytochemical compounds and toxicity test based on OECD guideline.

The results showed that the active phytochemicals present were saponin, flavonoids, tannin, steroids and cardiac glycosides in adequate amount. The toxicity present in the leaves finds scope to be experimented as botanical pesticides in agriculture farm as an alternative of chemical rodenticides.

Keywords: *phytochemicals, toxicity, extract*

Introduction

Phytochemicals are naturally synthesized in all parts of the plant body such as bark, leaves, stem, root, flower, fruits, seeds, etc. (Ugochukwu et al., 2013). These phytochemicals are non-nutritive plant chemicals but have protective or disease preventive properties. Plant produces these chemicals to protect itself where studies demonstrate that many phytochemicals can also protect humans against diseases (Poongothai et al., 2011). The quantity and quality of phytochemicals present in plant parts may differ from one part to another (reference needed). Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently in different parts of the world (Ugochukwu et al., 2013) and are involved in plants defense against aggression by pathogens. *Lyonia ovalifolia*, a member of the genus *Lyonia* belonging to Ericaceae family, has tannins, saponins, steroids, flavonoids, and cardiac glycosides and show antibacterial activities against bacterial pathogens (Negi et al., 2012 as cited in Acharya et al., 2014). *Lyonia ovalifolia* (anger) is deciduous tree. *L. ovalifolia* grows at an

altitude of about 1300-3300 masl in Nepal. The flowering period of *L. ovalifolia* is May to June and fruiting period is July to September (Fang & Stevens, 2005).

According to the farmers, newly sprouted leaves and buds are poisonous to animals such as young goats but in humans, the leaf juice is applied to treat scabies and itching (Manandhar, 2002). *L. ovaliafolia* (Wallich) has been used in a folk medicine for the treatment of wounds, cuts, burns, scabies, etc. by different local communities of Nepal (Acharya et al., 2014). This might be because of defensive mechanism of the plants by the production of secondary metabolites. The use of this plant can be beneficial in antibacterial activities. This planthas not yet been researched as pesticides, only it is known to have toxic effect on the animals (young goats). If the young leaves of the plant are really found to be toxic then the plant might be used as an alternative the chemical pesticide. Ministry of Agriculture and Livestock Development has been researching in the alternatives of the chemical pesticides because of its negative impacts in human as well as animal health, the degradation in environmental quality, reduction of soil fertility as well as banning and reducing the use of chemical pesticides and encouraging the use of different biological pesticides (G.C. 2015) so, this study might provide a way for a new generation of biological pesticides. Hence, this study is performed to study the toxicity level of the leaves and to test the leaves as an alternative of chemical rodenticide.

Materials and Methods

Study Area

The study was carried in Namobuddha. It lies in Kavrepalanchowk District and about 40km South East from the Kathmandu.

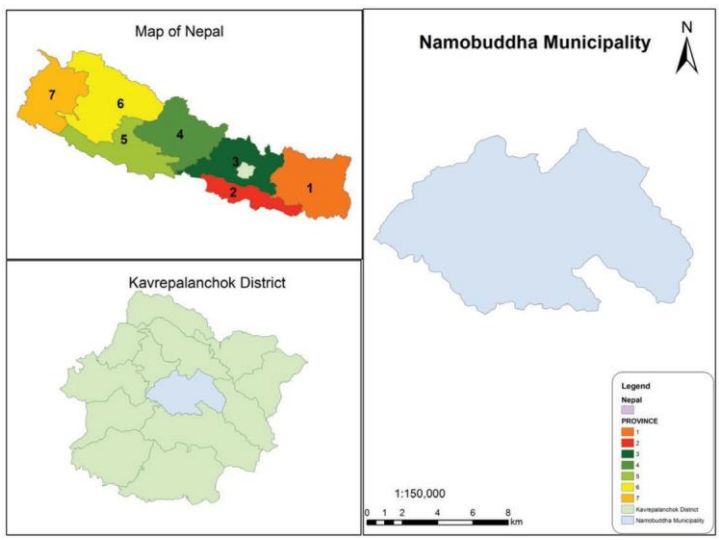

Figure 1: Study Area

Figure 2: Google Map of Study Area (Accessed on 2016)

Sample Collection and Identification

A random collection of young leaves were done in the month of March 2016 and identified as *L. ovalifolia* in National Herbarium and Plant Laboratories, Kathmandu.

Method of Extraction

The collected leaves were air-dried at room temperature under shade and grinded into fine powder. About 40 gms of the powder was taken in the percolator. It was filled with equal amount of distilled water and alcohol. The apparatus was allowed to settle. After an hour, it was shaken and kept for 24 hours. The solution was then poured in the beaker and was dried in water-bath.

Method of Treatment

1. Phytochemical Screening: Different processes were adopted for analyzing the types of phytochemical compound groups that are present in the leaves. The process adopted for the screening is shown below:

S.N.	Chemical Test	Process
1.	Test for Proteins (Millon's test)	3 ml extracts + 5 ml Million's reagent = White ppt. + warm ppt. = Brick red or the ppt. dissolves giving red colored solution (Ugochukwu et al., 2013).
2.	Test for Carbohydrates (Fehling's test)	(Fehling A + Fehling B reagents) + Few drop of extract + Boiled = Brick red colored precipitate (Joseph et al., 2013).
3.	Test for Phenols	Crude extract (500mg) + 5 ml of distilled water + Few drops of neutral 5% ferric chloride solution = Dark green color (Ahmad et al., 2013)
4.	Test for Saponins (Foam test)	Crude extract (500mg) + 5 ml of distilled water + Shaken vigorously = Formation of stable persistent foam (Kavishankar et al., 2011)
5.	Test for Tannins	Extract (2 ml) + 10% alcoholic $FeCl_3$ solution = Formation of blue or greenish color solution (Ugochukwu et al., 2013).
6.	Test for Steroids	Extract (1 ml) + Few drops of chloroform + acetic anhydride + conc. H_2SO_4 = Formation of dark pink or red colour (Ugochukwu et al., 2013).
7.	Test for Terpenoids	Extract (2 ml) + 1 ml of chloroform + Few drops of conc. H_2SO_4 = Reddish brown precipitate (Ugochukwu et al., 2013).
8.	Test for Flavonoids (Alkaline Reagent test)	Extract (2 ml) + Few drops of NaOH solution = Formation of intense yellow color + few drops of dilute HCl = Colorless (Minj et al., 2017).
9.	Test for Cardiac Glycosides (Keller kiliani Test):	Extract (2 ml) + 1 ml glacial acetic acid + Few drop 5% FeCl3 + 1 ml conc. H_2SO_4 = Brown ring on the interface (Minj et al., 2017).
10.	Test for Alkaloids	Extract (2 ml) + 1 ml of 1% HCl + heat gently + Few drops of Mayer's reagent and Wagner's reagent = Turbidity (Minj et al., 2017).

Toxicity: OECD Guideline (2001) for Testing of Chemicals (Acute Oral Toxicity Up and Down Procedure) was adopted for the test of toxicity. This method involves the sequential dosing of single animals (Swiss Albino Mice) with the test substance within a time interval of 48 hours. After the administration of the first dose, the next is determined by the outcome of the subsequent dose administered. If the animal survives the subsequent dose, the dose is adjusted upward, but when mortality is recorded at subsequent dose, it is adjusted downward. The adjustment of dose either upward or

downward is by a constant factor. Testing is terminated when the upper limit (2000-5000 mg/kg) have been reached without mortality or when the LD_{50} have been established from the test.

Acute Oral Toxicity Up and Down Procedure was divided into stages, with the outcome from each stage determine the next step to take (i.e., whether to terminate or proceed to the next stage).

i. Stage 1: This stage requires four animals (Swiss Albino Mice). These animals are divided into four groups of one animal each where the animals should be observed for 1 hours post-administration and then 10 minutes every 2 hours interval for about 24 hours. The behavioral signs of toxicity and also mortality should be recorded. Where no mortality is recorded at this stage, the testing should proceed to stage 2.

ii. Stage 2: This stage involves three animals, which are divided into three groups of one animal each. Different doses of the test substance (higher than those used in stage 1) are administered to the different animals and then observed for 1 hour after administration and periodically for 24 hours. Behavioral signs of toxicity and mortality should be noted. If no mortality occurred, testing should proceed to stage 3.

iii. Stage 3: This stage requires five animals. Various high doses of test substance (with 5000 mg/kg as the highest) are administered to the test animals. Observation is done for 1 hour after administration and then 10 minutes every 2 hours for 24 hours. Behavioral toxicity signs and also mortality should be recorded. This is the final stage of testing and where no mortality is recorded at this stage, the LD_{50} of the test substance is said to be greater than 5000 mg/kg and hence has a high degree of safety.

Initially the main test was performed for each sample assuming the sample was likely to be toxic. While testing the extract the test animal was kept fasting for 24 hours. The test animals weren't fed for 24 hours except water.

Calculation of the dose

Dose calculation is based on the OECD (2001). If 20gm test animal (Swiss Albino Mice) is to be given 175mg/kg then:

=175mg/kg

=1.75mg/10gm

=3.5mg/20gm

So, 3.5mg of the extract should be given to 20gm. Mice. Hence, 3.5gm of the extract

should be dissolved in l ml and 0.2 ml should be fed.

Results and Discussion

Phytochemical Screening

During the study, the color was observed for the confirmation of the presence of the phytochemicals. The dark color shows the presence of phytochemicals was adequate amount and light color shows the trace amount. Saponin, steroids, flavonoids, cardiac glycosides and tannins were present in adequate amount in the leaves of *Lyonia ovalifolia*. Acharya *et al.* (2014) studied the antioxidant and antimicrobial properties of Leaves *L. ovalifolia*. Also, studied phytochemicals present in the leaves using different extracts; alkaloids was found in appreciable amount in chloroform extract; tannins, phenols, carbohydrates & glycosides in appreciable amount and alkaloids in trace amount in ethanol extract. The difference in the phytochemicals might be due to the types of solvent used i.e. use of polar and nonpolar solvent in the extraction of the leaves. It showed that some of the chemicals in the plants are nonpolar.

Toxicity

Swiss albino mice were kept fasting for 24 hours (water was provided) and next day different doses were fed and observed for 48 hours. Since none of the mice died in the low concentration of the dose of the extract. The dose was increased to maximum following OECD (2021) until the dose was fatal.

Table 1: Toxicity Test of leaves of L. ovalifolia

S.N.	Dose (mg/kg)	Weight of Mice (gm.)	Results
1	175 mg/kg	27 gm.	Non-Lethal
2	550 mg/kg	25 gm.	Non-Lethal
3	1750 mg/kg	29 gm.	Non-Lethal
4	2300 mg/kg	26 gm.	Non-Lethal
5	2400 mg/kg	31 gm.	Non-Lethal
6	2600 mg/kg	28 gm.	Non-Lethal
7	2750 mg/kg	30 gm.	Non-Lethal
8	3100 mg/kg	27 gm.	Non-Lethal
9	3400 mg/kg	25 gm.	Non-Lethal
10	3750 mg/kg	27 gm.	Non-Lethal
11	4100 mg/kg	30 gm.	Lethal
12	5000 mg/kg	29 gm.	Lethal

Table 2: Confirmation of Toxicity

S.N.	Dose (mg/kg)	Weight of Mice (gm.)	Results
1	3750 mg/kg	35 gm.	Non-lethal
2	3750 mg/kg	32 gm.	Lethal
3	3750 mg/kg	34 gm.	Lethal
4	3750 mg/kg	29 gm.	Lethal
5	3750 mg/kg	28 gm.	Lethal

The result showed that the mice survived the dose of 3750 mg/kg and died at a dose of 4100mg/kg. A confirmatory test was performed for confirming whether the result obtained was correct or not assuming the acute toxicity must be between 3750 mg/kg-4000mg/kg. The confirmatory test supported the results.

The toxicity of the plant depends upon the types of phytochemicals present in the plants. Flavonoids and tannins are non-toxic. Saponins can sometimes be toxic and it is used for killing fishes because saponin accumulates in the gills of the fishes hence, blocks the air passage. Cardiac glycosides are mainly used to boost up cardiac muscle but the slight increase in the dose may cause the instability of the cardiac muscle hence giving a heart attack (Kassop et al., 2013). Also, Kassop et al., (2013) concluded that cardiac glycosides are naturally occurring toxins and is a major clinical problem in parts of the developing world causing a significant number of deaths each year.

Furthermore, Ganpisetti et al., (2016) found cardiac glycoside commonly used in the treatment of chronic heart failure (CHF), atrial fibrillation, and reentrant supraventricular tachycardia. The lethal dose of most glycosides is approximately 5-10 times the minimal effective dose and only about twice the dose that leads to minor toxic manifestations (citations needed). Bhandary et al., (2012) found Glycosides as naturally cardio-active drugs used in the treatment of congestive heart failure and cardiac arrhythmia. Steroids are also toxic to some extent.

Conclusion

The study showed that saponin, steroids, flavonoids, cardiac glycosides and tannins were present in adequate amount in the leaves of *Lyonia ovalifolia*. The presence of steroids and cardiac glycosides were found to be toxic for the plant of *L. ovalifolia*. The leaves could be regarded as lethal as observed during laboratory analysis since the toxicity value is greater than 2000 mg/kg i.e. the LD_{50} was greater than 2000 mg/kg. Different authors of different literatures also support that the presence of cardiac glycosides to be toxic even if given in slightly higher doses. Hence, *L. ovalifolia* indicated that this plant could be potent rodenticides.

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