

**PISCICIDAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS
OF EFFECTIVE HERBS OF NEPAL**



**By
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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.

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ABSTRACT

The freshwater fish *Clarias batrachus* (Linnaeus) of the family Clariidae locally known as Mangur is one of the stronger fish having accessory respiratory organs. Organophosphates and organochlorine poisons are commonly used for fishing is highly toxic stable compounds remain stored in fish tissue. In Nepal, many fish stupefying plants are used to stun fish that is comparatively safe for human consumption. Indigenous five plants are used for killing fish namely, *Agave americana* (Ketuke), *Budhleia asiatica* (Bhimsenpati), *Euphorbia royleana* (Siundi), *Madhuca indica* (Mahua), *Sapium insigne* (Khirro). These plants were collected from Kathmandu and Kapilvastu. Samples were air dried, powdered and extracted with 50% methanol by soxhlet method.

Comparative toxicity at 500 ppm concentration and toxic strength at different concentrations of most toxic herb was performed. Ten fishes were stocked to each of the experimental boxes and a control box in replicates and toxicity test lasted for every 24 hours. Based on the 24 hours lethal dose (LD₅₀) the plants extract with the toxicity to *C. batrachus* of the plants arranged in the order of decreasing toxicity as follows: *S. insigne* > *E. royleana* > *M. indica* and for 48 hours *B. asiatica* > *A. americana*.

The work also provides coverage of phytochemicals (alkaloid, tannin, saponin, flavonoid, steroid, cardiac glycoside, terpenoid and phenols) present in the plant samples. The chemical parameters after and before the extract application were analyzed to know the fluctuation in parameters of aquarium water. The quantity of oxygen is drastically decreased from 13.28 ppm to 2.81 ppm below the standard level after the application of extract and the ammonia level is increased from 0.6 ppm to 3 ppm beyond standard level. The mortality percent of fishes due to the application of herbal extract compared with Nuvan insecticide (62.5 ppm).

The results of the study showed that locally available plants in Nepal have potential to be used as piscicide which can be an alternative to harmful chemical piscicides.

LIST OF FIGURES

Figure 1.1 <i>Agave americana</i>	7
Figure 1.2 <i>Budhlea asiatica</i>	8
Figure 1.3 <i>Euphorbia royleana</i>	8
Figure 1.4 <i>Maduca indica</i>	9
Figure 1.5 <i>Sapium insigne</i>	9
Figure 3.1 Grating herbs for fish poisoning	15
Figure 3.2 Structural formula of Caffein and Ephedrin	17
Figure 3.3 Structural formula of Tannin	18
Figure 3.4 Structural formula of Solanin	19
Figure 3.5 Basic structure of Flavonoids	20
Figure 3.6 Molecular structure of Rotenone	21
Figure 3.7 Basic structure of Steroids	25
Figure 3.8 Molecular structure of Cardiac glycosides	25
Figure 3.9 Basic structure of terpenoid	26
Figure 3.10 Basic structure of Phenol	27
Figure 4.1 Study area	33
Figure 4.2 Weighing extract with the help of chemical balance	36
Figure 4.3 Soxhlet extractor	36
Figure 4.4 Drying extract with the help of rotatory vacuum evaporator	37
Figure 4.5 Drying extract in a dessicator	37
Figure 4.6 Testing herbal extracts on fish in a controlled condition at laboratory	38
Figure 4.7 Testing phytochemicals of herbal extract	43
Figure 5.1 Piscicidal strength of herbal extract on <i>Clarias batrachus</i> at 0.05% concentration	47
Figure 5.2 Toxic strength of Khirro (<i>Sapium insigne</i>) extract at different concentration on <i>Clarias batrachus</i> LD ₅₀ = 0.02375 %.	51

LIST OF TABLES

	Page No.
Table: 1. Geographical situation and estimated water surface area in Nepal.	3
Table: 2. Plants used as fish poison known in Nepal.	6
Table: 3. List of plant poison used for fish killing.	7
Table: 4. Showing the doses and effect of rotenone on different animals.	22
Table: 5. Major classes of phytochemicals with their mechanism of action.	31
Table: 6. Methodology of phytochemicals analysis of collected specimens.	42
Table: 7. Amount of extract obtained from the plant samples.	45
Table: 8. Piscicidal effect of different herbs at 0.05 % concentration on <i>Clarias batrachus</i> (Mangur) at 24 and 48 hours time standard.	46
Table: 9. LD ₅₀ values of the different test plants to <i>Clarias batrachus</i> at different durations.	49
Table: 10. Piscicidal effect at different concentrations of <i>Sapium insigne</i> extract after 24 hours.	50
Table: 11. Water quality analysis.	53
Table: 12. Phytochemical analysis of different plant species:	54

LIST OF ABBREVIATIONS

APHA	-	American Public Health Association
BOD	-	Biological Oxygen Demand
CITES	-	Convention on International Trade in Endangered of Wild Fauna and Flora Species
cm	-	Centimeter
DO	-	Dissolved Oxygen
DOFD	-	Directorate of Fisheries Development
E	-	East
eg.	-	Example
Fig.	-	Figure
ft.	-	Feet
gm.	-	Gram
ha.	-	Hector
IUCN	-	International Union of Conservation of Nature
Km.	-	Kilometer
Km ²	-	Square kilometer
m.	-	meter
mg.	-	milligram
mg/kg	-	Milligram Per Kilogram
mg/l	-	Milligram Per Liter
ml	-	Milliliter
mm	-	Millimeter
N	-	Normality
NAST	-	Nepal Academy of Science and Technology
NCL	-	Niclosamide
No.	-	Number
PD	-	Parkinson's Disease
ppb.	-	Part per billion
ppm.	-	Part per million
S	-	South
sps.	-	species
Sq. km.	-	Square Kilometer
VDC	-	Village Development Committee
W	-	West
°C	-	Degree Centigrade
%	-	Percent

CONTENTS

Page No.

Acknowledgements	
Abstract	
Contents	
List of tables	
List of figures	
List of photographs	
List of maps	
List of abbreviations	
CHAPTER I: INTRODUCTION	1-10
1.1 General Background	1
1.2 Water Resources of Nepal	2
1.3 Fishing Methods	3
1.3.1 Nets	4
1.3.3 Lure (Paso)	4
1.3.2 Diversion of River Channel	4
1.3.4 Bow and arrow (Ban Hanne)	4
1.3.5 Fishing Rod (Balchi Hanne)	4
1.3.6 Electro Fishing	4
1.3.7 Water Poisoning	5
a) Chlorinated Hydrocarbons	5
b) Organophosphates	5
1.4. Plants Used for Fish Stupefying	5
1.4.1 <i>Agave americana</i> (Ketuke)	7
1.4.2 <i>Buddleia asiatica</i> (Bhimsenpati)	8
1.4.3 <i>Euphorbia royleana</i> (Siundi)	8

1.4.4 <i>Madhuca indica</i> (Mahua)	9
1.4.5 <i>Sapium insigne</i> (Khirro)	9
1.5 Justification of the Study	10
1.6 Limitation of the Study	10
CHAPTER II: OBJECTIVES	11
CHAPTER III: LITERATURE REVIEW	12-31
3.1 Wild Use of Herbal Fish Poisoning	15
3.2 Biological Use of Phytochemicals	16
3.2.1) Alkaloids	17
3.2.2) Tannin	17
3.2.3) Saponin	18
3.2.4) Flavonoids	20
a) Rotenone (a class of isoflavones)	21
b) Rotenone toxicity	22
c) Toxic dose of rotenone	23
d) Fate of rotenone in natural water systems	23
e) Proposed link between rotenone use and Parkinson's disease	24
3.2.5) Steroids	24
3.2.6) Cardiac glycosides	25
3.2.7) Terpenoid	26
3.2.8) Polyphenols	26
3.3 Other Uses of Herbal Piscicides	27
3.3.1) Ketuke (<i>Agave americana</i>)	27
3.3.2) Bhimsenpati (<i>Budhleia asiatica</i>)	27
3.3.3) Siundi (<i>Euphorbia royleana</i>)	28

3.3.4) Mahua (<i>Madhuca indica</i>)	28
3.3.5) Khirro (<i>Sapium insigne</i>)	28
3.4 Analysis of Water Quality	29
3.4.1 Hydrogen Ion Concentration	29
3.4.2 Hardness	29
3.4.3 Ammonia	29
3.4.4 Dissolved Oxygen	30
3.4.5 Biological Oxygen Demand	30
CHAPTER IV: METHODOLOGY	32-43
4.1 Study Period	32
4.2 Sources of Data Collection	32
4.3 Study Area	32
Plant Collection	34
4.4 Phytochemical Tests	35
4.4.1 Soxhlet Extraction of Herbs	35
4.4.2 Toxic strength of herbal extracts	37
4.5 Chemical Analysis of Water	39
4.5.1 Hydrogen ion Concentration (pH)	39
4.5.2 Dissolved Oxygen	39
4.5.3 Total Hardness	40
4.5.4 Ammonia	40
4.5.5 Biochemical Oxygen Demand	41
4.6 Phytochemical Test of Herbal Extract	41
CHAPTER V: RESULTS AND DISCUSSION	44-52
5.1 Fish Poisonous Herbs and Their Wild Use	44
5.2 Herbal Extract	44

5.3 Ichthyotoxic Strength of Herbal Extract	45
5.4 Ichthyotoxic Strength of Khirro (<i>Sapium insigne</i>)	49
5.5 Chemical Analysis	51
5.5.1 Water	51
a) Hydrogen Ion Concentration	51
b) Dissolved Oxygen	52
c) Total Hardness	52
d) Ammonia	52
e) Biological Oxygen Demand	52
5.5.2 Plant Extract	53
5.6 Tested Phytochemicals	54
5.6.1) Alkaloids	54
5.6.2) Tannin	54
5.6.3) Saponin	55
5.6.4) Flavonoids	55
5.6.5) Steroids	55
5.6.6) Cardiac glycosides	55
5.6.7) Terpenoids	55
5.6.8) Polyphenols	55
CHAPTER VI: SUMMARY	56-57
CHAPTER VII: CONCLUSION	58-59
CHAPTER VIII: RECOMMENDATION	60

CHAPTER I

INTRODUCTION

1.1 General Background

Nepal, situated along the southern slopes of the Himalayan mountain range, is a fascinating landlocked country. It is situated between India to the south, east and west and the Tibetan region of China to the north. Its territory, which has an area of 54,462 square miles (147,181 Km²), extends roughly 500 miles from east to west and 90 to 150 miles from north to south. It is located between 80 °4' and 88° 12' longitude and between 26° 22' and 30° 27' latitude.

Fishes are well defined group of vertebrates. They inhabit all kinds of environment. They have a very special place in human history and civilization. Fishes are the primitive group of the cold blooded vertebrates with gills and fins. There are at least 27,800 species of fish in the world of which about 10,000 are fresh water fish species. These figures probably underestimate the true number, as more species continue to be described. Thus, there may be well over 35,000 species of fish world wide (Shrestha, 2008).

The knowledge of occurrence of fishes in Nepal dates back to the distant past. Hamilton was perhaps the first author to describe and record systematically fishes of Nepal in his admirable work "Fishes of the Ganges" in the year 1822.

From the recent taxonomic revision, Taft (1955) prepared a checklist of the fishes known from Nepal. His list contained 95 species, representing 13 families. Further De Witt (1960) elaborated the Taft's checklist by adding some new species. His list included 102 species representing 21 families. Majpuria and Shrestha (1968) published a paper on fresh water fishes and fisheries in Nepal. Similarly, Shrestha (1973-78) worked on fishes of Nepal and reported 114 species from this country. According to Shrestha (2007), there are 232 fish species in Nepal belonging to 98 genera, 35 families and 11 orders. Ng and Edds (2005) contributed several research papers on fishes of Nepal and described some new species also.

Fishes, during the course of their evolution, have become adapted to life in many kinds of aquatic environment, from the depth of seas to the creeks of snow fed

high mountain streams, from the tropical to circumpolar regions, even colonizing in salt water lakes and hot springs (Shrestha, 2008).

About 10 million people die every year in the world either by starvation or by malnutrition. Absence of protein or inadequate supply of protein in diet is one of the major factors causing malnutrition. It is estimated that between 15 and 20 percent of all animal proteins come from aquatic animals (Shrivastava, 1985).

In many countries, especially developing countries, the average per capita consumption may be low, but fish may be the staple food in coastal area and among the poor, and an important source of animal protein. Global production from capture fisheries and aquaculture supplied about 101 million tones of food fish in 2002, providing an apparent per capita supply of 16.2 kg (live weight equivalent), with aquaculture accounting for the growth in per capita supply since 2000. The demand for fish for food is expected to continue to grow. Based in projected population growth and on the maintenance of the present world level of consumption, by 2010 it could reach 120 million tones a year, a substantial increase over the 75 million to 85 million tones of the mid-1990s. Overall, fish provided more than 2.6 billion people with at least 20 percent of their average per capita animal protein intake (Anonymous, 2004). Asia, which combines a relatively high per capita consumption with large population, is by far the most important fish consuming region. Europe is the second largest food fish- consuming continent. Fish consumption is generally exceptions being found among the small developing island states. The lowest levels of consumption occur in Africa and the Near East (Anonymous, 1998).

According to K.C. (1999), about 75 percent of the world fish catch is used for human consumption. In Nepal, fish production from culture practices and capture fisheries reached 48750 mt in 2008/2009. The per capita fish production is reported to be 1700 gm and projected to reach 3000 gm by the year 2018 (DOFD, 2008/2009).

1.2 Water Resources of Nepal

Rivers in Nepal covers an area about 395,000 hectares. A number of relatively small to medium sized lakes occur in various parts of Nepal covering an area of 5,000 hectares and numbers of relatively small reservoirs have been constructed, which

covers an area of about 1,500 hectares. There are 51,300 hectares of marginal swamps and paddy fields (DOFD, 2001/2002).

It is estimated that there are altogether 6000 rivers totaling 45,000 km. in length. Out of 6000 rivers, approximately 1000 of these are more than 10 km. long and about 100 of them are longer than 160 km. (Sharma, 1997). The Koshi, Gandaki and Karnali are the main river systems receiving a principal share of their water supplies from the snow melt glaciers and small tributaries. The total area drained by these rivers is more than 60 % of the total drainage area of the country. These three main river systems are originating from the northern slope of the Greater Himalayas consisting seven tributaries covering large catchments area. Besides these big rivers, there are many more medium and small sized rivers and streams.

The estimated water resources and its area covered are explained in the following table:

Table: 1. Geographical situation and estimated water surface area in Nepal.

S.N.	Resource Details	Estimated Area (ha)	Coverage percent	Potential for Fisheries (ha)
1.	Natural Waters	401500	48.8	–
1.1.	Rivers	395000	48.0	–
1.2.	Lakes	5000	0.6	3500
1.3.	Reservoirs	1500	0.2	78000
2.	Village Ponds	6735	0.8	14000
3.	Marginal Swamps around irrigated paddy fields	12500	1.4	12500
4.	Irrigated Paddy Fields	398000	49.0	100000
	Total	818500	100	208000

Source: Directorate of Fisheries Development (DOFD) 2001/2002.

1.3 Fishing Methods

The waters of the Koshi, Gandaki, Karnali and Mahakali rivers and rivulets provide excellent opportunities for the peoples interested in angling in Himalayan waters of Nepal. Fishing in these clear waters, one and same kind of fishing gears

may not be applicable to fish all round the year. A large number of fishing implements have been devised to suit the water resources.

Important fishing implements include cast net (Jal), gill net, bamboo fish trap (Dhadia), rod and line, lure (Paso), scooping net (Ghorleng), lift net (Thakauli) etc.

1.3.1 Nets

A net is a piece of webbing, in which the twines are intersected into rectangular meshes, gives a certain form. Fine nylon threads and small metallic weights of iron or leads are used to prepare a net. Cast net, gill net and tiyari are the examples of nets used in Nepal.

1.3.2 Diversion of River Channel

In low water phase fishermen divert small side branches of the river from one place to other, thus creating practically fishing channel to scoop fish.

1.3.3 Lure (Paso)

During low water phases (January to April), a special kind of baited nylon loop are used to trap fish. A line made up of small bamboo shoot is set in mid-stream together with sink, a loop and bait. Fish entering the baited loop may be tied automatically along.

1.3.4 Bow and arrow (Ban Hanne)

This method is applied where the water level is low. It is made up of by thin iron rod, making it a shape of arrow. It functions like arrow. This method is used to catch *Gagata*, *Channa* etc.

1.3.5 Fishing Rod (Balchi Hanne)

It consists of three parts: Hook, line and rod. Hook (Balchi) is prepared generally by the fisherman from the rims of umbrella, but now a days it is available readymade in the market. Fine nylon thread is tied at curved tip of the rod and a hook at the distal end of thread. Different types of bait are fixed to the hooks.

1.3.6 Electro Fishing

In this method, the fish respond to polarity of electric fields and swim towards the electrode with increasing potential. If two electrodes are put into water, the fish starts swimming towards the positive pole, while the current was on. Thus a large

number of fish can be easily caught by placing anode in the fishing net, and the cathode near the boat.

1.3.7 Water Poisoning

Poisons are commonly used for fishing in different water bodies as the application of poison is the easy method of fishing. Poisoning should be done with caution to avoid harmful effects to human beings, livestock and the surrounding environment (Shrestha, 1997).

Fishes are killed by poisoning with chemicals viz. aldrin, dieldrin, endrin, DDT, thiometon, phosphamidon dichlorovinyl-phosphate (DDVP) etc. Wide varieties of chemical poisons are available and can be grouped as follows:

a) Chlorinated Hydrocarbons:-

The common chlorinated hydrocarbons used for the fish poisoning are:

- i) Aldrin ($C_{12}H_8Cl_6$) 0.2 ppm.
- ii) Dieldrin ($C_{12}H_8Cl_6O$) 0.01 ppm.
- iii) Endrin ($C_{12}H_8Cl_6O$) 0.01 ppm.

The solutions of these chemicals prepared with water are sprayed over the water surface.

b) Organophosphates:-

Organophosphates are generally less toxic to fish but adversely affect other aquatic biota. The common compounds used are Thiometon, Phosphamidon and Dimethyl dichlorovinyl-phosphate (DDVP). These are used at a concentration of 2-20 ppm; depending on the density of fish (Santhenum et al, 1987).

1.4 Plants Used for Fish Stupefying

Fish stupefying plants have historically been used by many hunter gatherer cultures to stun fish, so that the fish become easy to collect by hand. Some of the important Indian plants used for fish poisoning are, e.g.; Azalea (*Rhododendron species*), Black locust (*Robinia species*), Blue cardinal flower (*Lobelia siphilitica*), Buttercup (*Ranunculus species*), Caladium (*Caladium xaitliosoma*), Cardinal flower (*Lobelia cardinalis*), Castorbean (*Ricinus communis*), Chokecherry/Cherry (*Prunus species*), Climbing nightshade (*Solanum dulcamara*), Clover (*Trifolium species*), Marsh marigold (*Caltha palustris*), Philodendron (*Philodendron species*), Pigweed (*Amaranthus species*), Poison hemlock (*Conium maculatum*), Ponderosa pine (*Pinus ponderosa*), Potato (*Solanum tuberosum*), Red maple (*Acer rubrum*), Rhubarb (*Rheum rhaponticum*), Horsechestnut (*Aeseulus species*), Iris (*Iris species*-Bulb),

Jack-in-the-pulpit (*Arisaema species*), Jimsonweed (*Datura species*), Lily-of-the-valley (*Convallaria majalis*), Sweet pea (*Lathyrus species*), Tobacco (*Nicotiana tabacum*), White snake root (*Eupatorium rugosum*), Wild bleeding heart (*Dicentra species*) (Begley, 2000).

Derris roots powder of *Derris trifoliata* (5-10 ppm) is commonly used for killing unwanted fishes. The required quantity of derris powder is dissolved in water in a container to make a thick brown solution and is spread all over the water surface by dipping out with a cup and throwing. The water surface is splashed to ensure quick and effective mixing of the poison in water evenly. The other plants poison which may be used are the powdered seeds of *Croton tiglium* (5 ppm), root powder of *Milletia pachycarpa* (5 ppm), seed powder of *Barringtonia actuangula* (15 ppm), unripe fruit powder of *Randia dumetorum* (10 ppm) and bark powder of *Walsuria piscidia* (10 ppm). Besides these, other indigenous plant poisons used for killing fish in different part of the country are listed in Table- 2.

Table: 2. Plants used as fish poison known in Nepal.

S.N.	Local name of plant	Scientific name	Family	Parts of the plant used as a fish toxicant
1.	Surti	<i>Nicotiana tobacum</i>	Solanaceae	Leaf
2.	Bhang, Ganja	<i>Cannabis sativa</i>	Cannabinaceae	Leaf
3.	Bhut Kanda	<i>Solanum xanthocarpum</i>	Solanaceae	Fruit
4.	Bel	<i>Aegle marmelos</i>	Rutaceae	Bark
5.	Chiuri	<i>Madhuca butyraceae</i>	Sapotaceae	Seed (oil cake)
6.	Neem	<i>Azadirachta indica</i>	Meliaceae	Bark
7.	Saj	<i>Terminalia tomentosa</i>	Combretaceae	Bark
8.	Simal Tarul	<i>Manihot esculenta</i>	Euphorbiaceae	Root bark, whole plant
9.	Pirrae	<i>Polygonum hydropiper</i>	Polygonaceae	Whole plant
10.	Karam	<i>Adina cordifolia</i>	Rubiaceae	Bark
11.	Mainkanda	<i>Randi dumetorum</i>	Rubiaceae	Leaf, bark, flower and fruit
12.	Bhalayo	<i>Somecarpus anacardium</i>	Anacardiaceae	Leaf, bark, flower and fruit
13.	Khair	<i>Acacia catechu</i>	Leguminoceae	Bark
14.	Sirris	<i>Albizia lebbek</i>	Leguminoceae	Bark
15.	Satyasal	<i>Shorea robusta</i>	Dipterocarpaceae	Bark

Source: Shrestha and Jha (1993).

The following table indicates the plant species with their scientific name included in the investigation and the plant parts used for fish poisoning. The leaves of *Agave americana*, *Sapium insigne* and *Budhlea asiatica*, cake of *Madhuca indica* and all parts of *Euphorbia royleana* are used as fish poison.

Table: 3. List of plant poison used for fish killing.

S.N.	Common Name	Scientific Name	Family	Parts used
1.	Siundi	<i>Euphorbia royleana</i>	Euphorbiaceae	All parts
2.	Ketuke	<i>Agave americana</i>	Agavaceae	Leaves
3.	Khirro	<i>Sapium insigne</i>	Euphorbiaceae	Leaves
4.	Bhimsenpati	<i>Budhlea asiatica</i>	Budhleiaceae	Leaves
5.	Mahua	<i>Madhuca indica</i>	Sapotaceae	Cake

The present investigation is focused on these five plants namely *A. americana* (Ketuke), *B. asiatica* (Bhimsenpati), *E. royleana* (Siundi), *M. indica* (Mahua) and *S. insigne* (Khirro) and their descriptions are given below:

1.4.1 *Agave americana* (Ketuke)

Family: Agavaceae

As originally described by Gentry (1970), Agavaceae consists of 18 genera and a little over 400 species, many of them native to western North America. One of the most familiar species is *Agave americana*, a native of tropical American. Common names includes century plant, Maguey (in Mexico), or American Aloe (it is not, however, closely related to the genus Aloe).



Fig. 1.1: *Agave americana*

This plant is locally known as ‘Ketuke’ in Nepal. The name “Century Plant” refers to the long time the plant takes to flower, although the number of years before flowering occurs depend on the vigor of the individual, the richness of the soil and the climate;

during these years the plant is storing in its fleshy leaves the nourishment required for the effort of flowering. Agaves can cause severe dermatitis (Shrestha, 1991). The juice of the more virulent agaves has been used as fish poison and arrow poison (Dimmitt, 2001).

1.4.2 *Buddleia asiatica* (Bhimsenpati)

Family: Buddleiaceae

Buddleia asiatica (Lour, 1981), Buddleiaceae is habitated to East Asia-India, Nepal to the Philippines. It is locally called ‘Bhimsenpati’ in Nepal. It is in leaf all year, in flower from January to April. The flowers are dioecious (individual flowers are either male or female, but only one sex is to be



Fig 1.2: *Budhleia asiatica*

found on any one plant so both male and female plants must be grown if seed is required) and are pollinated by Bees, Lepidoptera. The plant is not self-fertile. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils, requires well-drained soil and can grow in nutritionally poor soil. It can tolerate atmospheric pollution. The dried and powdered root is used in the preparation of fermented liquor (Facciola, 1990). The plant has been used as an abortifacient and also in the treatment of skin complaints (Chopra; Nayar and Chopra, 1986). The juice of the plant is applied as wash to treat skin diseases. The leaves and flower are used in worship and extract of leaf is used as fish poison (Manandhar, 2002).

1.4.3 *Euphorbia royleana* (Siudi)

Family: Euphorbiaceae

The plant *Euphorbia royleana* (Russell, 1997), Euphorbiaceae is erect, succulent shrub with whorled branches, 4 or 5 ridged, the spines paired and pointing down ward leaves large. The plant is locally known as ‘Siundi’ in Nepal. All parts of the



Fig 1.3: *Euphorbia royleana*

plants are poisonous, characterized by dermatitis, eye irritant, nausea, vomiting and diarrhea when ingested. Redness, swelling and blisters appear after contact with skin. This species has irritant properties Kinghom and Evans (1975) demonstrated the irritancy of the latex in a mouse ear irritancy assay and subsequently reported the presence of ingenol esters in the plant (Hardin; 1997).

1.4.4 *Madhuca indica* (Mahua)

Family: Sapotaceae

The two major species of genus *Madhuca* found in Nepal are *Madhuca indica* (Syn. *Brassica latifolia*) and *Madhuca longifolia* (Syn. *Brassica longifolia*). Mahua is the widely accepted as local name for the both these species. The plant is common in deciduous forests. *M. indica* is found mostly in Southern part of the country. *M. longifolia* is evergreen or semi-evergreen. The tree attains height up to 21 to 23 meters in 15 years. It fruits up to 60 years. It flowers at the end of February to April, in clusters at the end of branches, aromatic, fleshy, sweet petals, shed on ground, are collected for distillation of liquor or essential oils. Fruits appear in May–June, later in South and fall in April to July in North, August to September in South. The saponin in the cake is a powerful fish poison (Dahl, 1985).



Fig: 1.4 *Madhuca indica*

1.4.5 *Sapium insigne* (Khirro)

Family: Euphorbiaceae

About 120 species are found in tropical and subtropical regions and in America south to Patagonia. *Sapium insigne* locally known as 'Khirro' is a deciduous tree (about 10 m high) distributed throughout Nepal, India, Sri Lanka, China and Southeast Asia. It seems probable that the species of *Sapium* differ in their properties that some may be harmful and others are innocuous. The tree yields an acrid, vesicant milky juice also referred to its irritant properties. Members of this



Fig: 1.5 *Sapium insigne*

genus are not invariably hazardous. In Mexico, the milky sap is reputed to be harmful and it is reported on good authority that the Indians utilized it for poisoning their arrows. The sap is claimed to be poisonous and blistering in effect if in contact with the skin (Taylor et al, 1996).

1.5 Justification of the Study

Fishing with herbal poison is one of the important poisoning methods of the water body. Use of the fish poisons is very old practice in the history of mankind. Many hunter gatherer cultures use poisonous plants to stun fish so that they become easy to collect by hand. Some of these poisons paralyze the fish; others are thought to work by removing oxygen from the water. The poisons affect the fish on nervous, circulatory and/or respiratory systems. It is thus very important to study the effect of toxicity on human health also. Chemical analysis of poisonous herbs is important to identify the chemical nature of the poison constituent and their effective strength on human health. All over the globe, indigenous people use various fish poisons to kill the fishes. Although, very little work on fish poisonous herbs, their working mechanism, effectiveness on fishes has been done in Nepal.

Therefore, the present study entitled “**Piscicidal Activity and Pytochemical Analysis of Effective Herbs of Nepal**” has been undertaken to collect information health would be discussed. This study will open the mystery of use and disuse of the herbs as fishing poison. Moreover, it is also believed that the work will certainly provide necessary information for any further studies and research works for further pathological plants including these herbs.

1.6 Limitation of the Study

The experiments and lab works have been carried out in Natural Product Research Laboratory of Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur. The present study deals with the analysis of important chemical constituent of toxic herbs. The biochemical composition of poisoned fish and the cause of effectiveness and ineffectiveness on human health was also interesting part in the study. The separation and purification of pure toxic chemicals and their test on fishes of different species could not be undertaken due to limited time.

CHAPTER II

OBJECTIVES

The general objective of the present investigation is to study the fish poisonous herbs and their chemical constituents.

The specific objectives are as follows:

Specific objectives:

- Study of fish poisonous herbs and their wild use.
- Extract the herbal component and analyze the chemical composition.
- Find out the herbal toxic strength and
- Study the effectiveness of the herbs on fish.

CHAPTER III

LITERATURE REVIEW

Use of the fish poisons is very old practice in the history of human kind. In 1212 AD King Frederick II prohibited the use of certain plant piscicides, and by the fifteenth century similar laws had been decreed in European countries as well. All over the globe, indigenous people use various fish poisons to kill the fishes although very little literatures are available. Some of the fish stupefying plants are being used since long time by local people are recently well tested by many workers and are found to have many important medicinal properties.

Schmidt (1994-2009), studied that the bark of *Sebastiania ramirezii*, *S. pringlei*, *Sapium appendiculatum* and *S. biloculare* has piscicidal activity (Pennington, 1958 and Altschul, 1973), *S. aubletianum* (Burkill, 1935) and *S. insigne* (Taylor et al; 1983), latex is irritant while *S. laurocerasum*, *S. macrocarpum* and *S. madagascariense* milky juice is very poisonous to the skin (Lipp, 1982, Altschul, 1973, Watt and Brandwijk, 1962). The book “**Neurobehavioral Toxicity in Fish**” by Edward and Sandra (2010) focuses on a specific target organ or physiological system and describes how various agents disrupt the normal physiological systems and processes. Two case reports about hallucinatory fish poisoning (Luc and Philip, 2006) are published in “**Clinical Toxicology**”. Daniel and William (2010), provides coverage of toxic effects in the central nervous, immune, neurobehavioral and reproductive systems as well as describing general mechanisms of toxicity in “**Target Organ Toxicity in Marine and Freshwater Teleosts Systems**”. Dimmitt (2000), states the beneficial uses and toxicological property of family-Agavaceae in the article “**A Natural History of the Sonoran Desert**”. He explains the edible and medicinal uses of the *Agave*. Similarly, “**Agave Plant Health Benefit**” a research paper by Sahelian (2008) explains the phytochemical analysis and anti-allergic study of *Agave*. Bani et al. (2000) in their article “**Anti-inflammatory activity of the hydrosoluble fraction of *Euphorbia royleana* latex**” reported the anti-inflammatory and anti-arthritic effects of *E. royleana* in rats and mice. Deutsch (1988-2006), documented a concise overview of the contact-poisonous plants in his research work “**Contact-Poisonous Plants of the World**”. The first part of the paper briefly introduces the active principles, effects, treatment and geographical distribution. The second part

lists about 35 important plant species and describes them in detail. Fafioye (2005) documented some Nigerian piscicidal plants with known active ingredients with the view of ensuring their further development and conservation in the research paper **“Plants with Piscicidal Activities in Southwestern Nigeria”**. Ambedkar and Munian (2009) studied the Piscicidal activity of methanolic extract of *Capparis stylosa* on the freshwater fish *Channa punctatus*. A list of poisonous plants is created by Begley (2000) and Triplett (1997) under the title of **“Poisonous plants can be a hazard to pets, including fish”**. All plants listed are poisonous in various degrees to humans and pets including fish. Some plants are mildly toxic, while others can cause death. ([http:// cal. nbc. upenn. edu/ poison/ ppstcommon. htm](http://cal.nbc.upenn.edu/poison/ppstcommon.htm)).

Use of the herbal fish poisons has been documented in a number of sources involving catching fish from fresh and sea water (Dahl, 1985). Adrian and Jimmie (1998), reported the toxic property of *Sapium insigne* and *Euphorbia royleana* in their book **“Enjoy Trees”**, a guide to some attractive trees and shrubs found in Nepal. Prajapati, Purohit, Sharma and Kumar (2004), reported the chemical constituent of *Madhuca indica* in their book **“A hand book of medicinal plants”**. *Carreya arborea* is well-tested plant used as analgesic (Ahmed et al; 2002) and anti-diarrhoeal (Rahman et al; 2003). Some of the plants like *C. collinus* are a traditional poison used in the different part of the India (Sarathchandra and Balkrishnamurthy 1997, Thomas et al; 1991). Recent studies by Raghupati et al; (1992) revealed that a toxic compound cleistanthin B (2mg/ml) is reported from the *Cleistanthus collinus* which might be responsible for its toxicity for fishes. In addition, in earlier studies by Islam et al; (2002), it was revealed that the steam bark extracts of *Lannea coromandelica* caused lysis of cell membrane followed by fragmentation of cellular materials. (“[http://en. wikipedia. org/wiki/Fish toxins](http://en.wikipedia.org/wiki/Fish_toxins)”).

Shubhangi, Pawar, Patil and Patil, (2004), scientifically confirmed the toxic property of *Aesculus californica*, *Olax*, *Careya coccinea*, *C. arborea*, *Cleistanthus collinus*, *Lannea coromandelica*, *Banksia speciosa*, *Madhuca indica*, *Pterocarpus marsupium*, *Chloroxylon swietenia* for fish stunning in their research paper **“Fish stupefying plants used by tribals of North Maharastra”**. Chopra, Nayar, and Chopra (1986), in their **“Glossary of Indian Medicinal Plants”** research paper states the *Budhlea asiatica* plant has been used as an abortifacient and also in the treatment of skin complaints. Similarly, Manandhar (2002), in the book **“Plants and People of**

Nepal” explained that the juice of the plant is applied as a wash to treat skin diseases. The leaf is used in worship and the extract is used as fish poison. Karki and Rai (1982), studied the poisoning constituents of *Sapium insigne* and other plants used as fish poison, in the paper **“Observations on the effectiveness of some local plants used as fish poison”**. Singh and Agrawal (1984) reported that the latex of *Euphorbia royleana* has high molluscicidal activity in their paper **“Alteration in biogenic amine levels in the snail *Lymnaea acuminata* by the latex of *Euphorbia royleana*.”** Similarly, Tiwari and Singh (2004) reported the toxicological action of *E. royleana* in their research paper **“Piscicidal and anti-acetylcholinesterase activity of *Euphorbia royleana* stem bark extracts against freshwater common predatory fish *Channa punctatus*”**. An Indian Journal of Traditional Knowledge **“Herbal fish toxicant used by fishers of Karbi-Anglong district, Assam”**, Kalita, Dutta and Chaudhary (2005) studied and reported the use of plant, *Polygonum hydropiper* (Smartweed) as fish toxicant for catching fish from natural aquatic resources as well as for removal of uneconomical fishes from the aquaculture pond.

Tribal people historically used various plants for medicinal and food exploitation purposes. Singh (2007) extends the use notion for herbal fish stupefying plants. Bhattra, Karki and Mandal (2008), reported the medicinal value of many plants including *Euphorbia royleana* in the **“Medico-ethnobotanical study”**. Shrestha (1994) reported the fishing methods using herbal poison in her book **“Fishes, Fishing implements and methods of Nepal”**. Shrestha (1991) studied the medicinal value of *Agave americana* of Kathmandu Valley. Similarly, Shrestha (1973-78) worked on **“Fishes of Nepal”** and reported 114 species from this country. Shrestha (2003) reported that the rhizome and leaves of wild ginger (*Zingiber sp.*), bark of Kaphal (*Myrica esculenta*), the rind of Okhar or walnut (*Juglans regia*), Pirija (*Polygonum*), wild apple fruit (*Malus sp.*), are fish poisons and paralyze the fish making them semi-blind. Similarly, the book **“Fish Catching in the Himalayan Waters of Nepal”** and **“Ichthyology of Nepal”** by Shrestha (1995, 2008) also reports about the herbal fish poison. Medicinal properties of some plants including *Euphorbia royleana* and *Madhuca indica* are reported by Baral and Kurmi (2006) in their book **“A Compendium of Medicinal plants in Nepal”**. Joshi and Joshi (2001), Rajbhandari (2001), Manandhar (2002) listed medicinal plants including *Euphorbia* used in traditional phytotherapy as for disease and other conditions. In the list

Euphorbia hirta is described as asthma cure/bronchitis and gastrointestinal disorder. Phytochemical screening of 47 Nepalese medicinal plants belonging to 45 genera and 35 families was carried out in the research paper “**Phytochemical screening of some species of Nepalese Medicinal Plants**” by Gyawali, Jnawali and Kim (2008). *Agave cantula* leaf juice and root pastes are described as used in cuts and wounds (Rajbhandary and Ranjitkar, 2006). Medicinal value of *Euphorbia royleana* and *E. hirta* is reported by Dhama (2006), Joshi (2008), Burlakoti and Kunwar (2006) in their case studies in Kanchanpur, Western Terai, Darchula and Mahakali watershed area of Nepal (Jha et al; 2008).

3.1 Wild Use of Herbal Fish Poisoning

The mechanically achieved narcosis of fish is however, for less important than that produced chemically by so-called fish poisoning. For this purpose poisons, usually originating from plants are crushed, cut to pieces or pulverized and sprinkled on the water or added to bait. The fish are thereby narcotized or at least so affected that they rise to the water’s surface for air and can be captured easily. A necessary condition, however, is that this fishery shall take place in smaller areas of stagnant or, at any rate, not too rapidly running water. These piscicidal plants are used on freshwater aquatic ecosystem between October and January (dry season) and after the first rainfall of the year. Fish poisoning is generally used in small bodies of water, up to about 500 hectares, or to bays and arms of larger lakes. In running water barriers are installed downstream from the place where the poison is to be used in order to catch the more or less floating helpless fish.



Fig. 3.1: Grating herbs for fish poisoning.

To make this fishery more profitable the fish are driven together in certain sections of break or lake before the poisons is applied. In order to catch larger fish in deeper water, poisoned mixtures are put into the bellies of bait fish. As soon as the larger fish have taken this bait the poisoning effect makes them rise to the surface (Brandt, 1972).

The juice of the more virulent agaves has been used as fish poison and arrow poison (Dimmitt, 2002). Similarly, the leaf extract of *Budhlea asiatica* and bark and leaf of *Sapium* are widely used as fish poison. The latex of *Euphorbia* is less toxic to fishes like *Danio rerio*, *Poecilia reticulata*. After the removal of the oil from seeds of *Madhuca*, remaining cake is used for fish poisoning. It is very effective poison and fishes die due to its application (Manandhar, 2002).

Shrestha (2003) states that, the fish poisons are prepared from the stem, bark and fruit of many juicy or latex yielding plants. Timur (*Xanthoxylum alatum*), bark of Kaphal (*Myrica esculenta*), stem of the Titaepati (*Artimesia vulgaris*), bark of rind of walnut (*Juglans regia*), stem and root of Aryli (*Edgeworthia*), Chilly powder (*Caspicum*) are also used as fish poison.

The polygonum plants cause many side effects on fish. Fish become semi-blind and restless. They jump vigorously and lost their balance. After half an hour the fry and fingerlings are paralyzed due to neurotoxic effects of the poison. The poison solution is also released in deep pools and crevices with constant stirring. Most of fish in deeper water get suffocated due to the effect of poison in vital parts of gills, barbells, buccal cavity. They come up the surface and are scooped up easily with the help of dip net, lift nets, scoops and baskets. They wriggle out restlessly from one place to the other. The entire stretch of the dry channel below the stone dam is searched for fish, shrimp, crabs and tadpoles. When fishing activity is over, the stone dam is destroyed and the dry river bed is flushed with water. Finally, the fish trap set down-stream is taken out and fishes are collected. The collected fish are distributed among the participants according to certain tribal rules (Shrestha, 2003).

3.2 Biological Use of Phytochemicals

Plant extracts are referred to as botanicals and when poisonous to fish are called piscicides (Burkill, 1985). Such piscicidal plants contain different active ingredients known as alkaloids such as nicotine, pyrethrum, ryania, rotenone,

coumerin, resin, akuammine, tannins, saponins and diosgenin (Wang and Huffman, 1991). However, these alkaloids are toxic to fish and other aquatic organisms at high concentrations and wear off within a short time (Crandall and Goodnight, 1962; Olaifa et al., 1987; Kulakkattolickal, 1987; Adewunmi, 1990). Structural formula and biological use of the analyzed phytochemicals are given below:

3.2.1 Alkaloids

Alkaloids (meaning alkali-like) are an important class of basic organic compounds that occur in higher plants. They are known to contain one or more nitrogen heterocyclic rings as an integral part of their structure. An alkaloid is a compound of plant origin which has a structure containing one or more basic nitrogen heterocyclic rings, and can cause marked physiological activity in humans (Bahl and Bahl, 1996).

They produce striking physiological effects when administered to humans. Thus, some alkaloids stimulate the central nervous system, while others cause paralysis; some alkaloids act as pain relievers, others as local anesthetics; and still others act against infectious microorganisms. Most alkaloids are toxic and yet many find use in medicine. Initial signs and symptoms may be followed by hyperthermia, confusion, agitation, combativeness, seizures, coma and death (Richard et al, 2009).

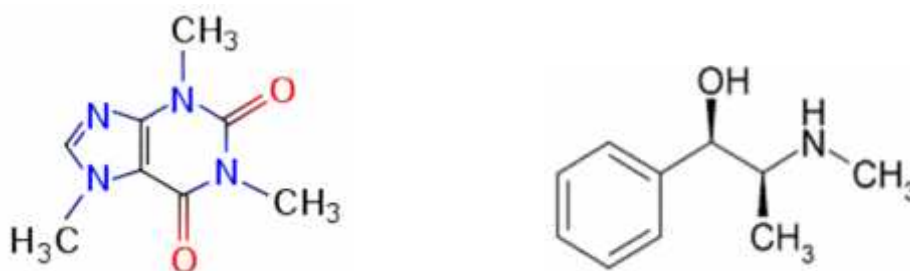


Fig. 3.2: Structural formula of Caffeine and Ephedrine.

3.2.2 Tannin

Tannins have molecular weights ranging from 500 to over 3,000. The term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules (Bate-Smith and Swain, 1962).

The term tannin (from *tanna*,) refers to the use of wood tannins from oak in tanning animal hides into leather; however, the compounds are widely distributed in many species of plants, where they play a role in protection from predation and perhaps also in growth regulation (McGee and Harold, 2004).

Tannins are used indirectly as molluscicides to interrupt the transmission cycle of schistosomiasis. They have also been reported to have anti-viral effects. When incubated with red grape juice and red wines with a high content of condensed tannins, the poliovirus, herpes simplex virus, and various enteric viruses are inactivated. Tannins can also be used to pull out poisons from poison oak or from bee stings, causing instant relief. The tannins help draw out all irritants from the skin because tannin is an astringent that tightens pores and pulls out liquids (Bajaj, 1988).

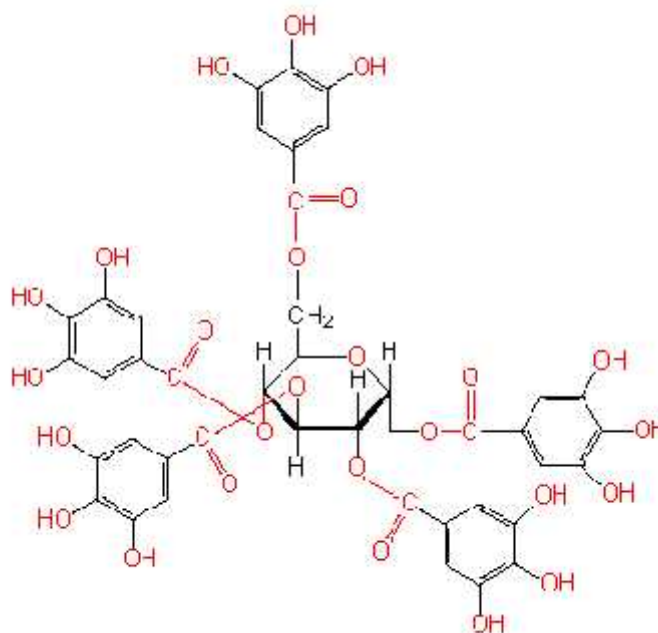


Fig. 3.3: Structural formula of Tannin.

3.2.3 Saponin

Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species.

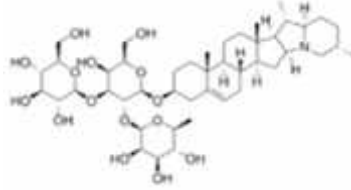


Fig. 3.4: Structural formula of Solanin.

Saponins normally break down in the digestive system and must enter the bloodstream to be toxic, but fish take in saponins directly into their bloodstream through their gills. The toxin acts on the respiratory organs of the fish without affecting their edibility. Saponins also cause the breakdown of red blood cells that help the toxin to spread quickly. Even though the effects of the poison are powerful, they are not usually fatal. Fish that are washed away into untainted water revive, and can return to their pre-toxic condition. Because of this, the fishermen would have to gather the stunned fish quickly as they floated to the surface.

Saponins are one of a group of glucosides found in many plant species with known foaming properties when mixed with water. Saponins lower the surface tension of water allowing the formation of small stable bubbles. The amount of foam created by a crushed plant sample, shaken with water in a jar, is a good indication of the amount of saponins present.

One research use of the saponin class of natural products involves their complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection (Francis, et. al. 2002). In addition, the amphipathic nature of the class gives them activity as surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes. Saponins have also been used as adjuvants in vaccines (Aldrich, 2009).

In plants, saponins may serve as anti-feedants and to protect the plant against microbes and fungi. Some plant saponins (e.g. from oat and spinach) may enhance nutrient absorption and aid in animal digestion. However, saponins are often bitter to taste, and so can reduce plant palatability (e.g. in livestock feeds), or even imbue them with life-threatening animal toxicity. Data make clear that some saponins are toxic to cold-blooded organisms and insects at particular concentrations (Foerster and Hartmut 2006).

Saponins are used on injection, for which it has a pharmacological reputation. It results in the lysis of the blood cells, haemolysis, like all detergents, and is therefore highly toxic. Saponins base are the basic of many arrow poisons.

The useful topical effects that all saponins have been widely neglected in modern pharmacology. The most noticeable effect is on the respiratory system, by reflex stimulation of the stomach wall brought about by a stimulating expectoration. There is an emetic effect when saponins are taken in bulk: elimination on the portions of the stomach gets promoted due to their detergent action. Intake of sub-emetic dosages, sublimates the emetic action to a reflex-stimulating expectoration.

Other saponins actually aid the absorption of important minerals and cause lesser irritating effect on digestive system.

Saponins have been used in modern times in the manufacture of fire extinguisher foam, toothpaste, shampoos, liquid soaps, and cosmetics and to increase the foaming of beer and soft drinks (Meta Cyc. Pathway, 2009).

Plant Families that contain significant saponins are: Amaryllidaceae, Convolvulaceae, Dioscoreaceae, Lamiaceae, Lecythidaceae, Liliaceae, Loganiaceae, Meliaceae, Menispermaceae, Papilionaceae, Solanaceae, Sapindaceae, Sapotaceae, Scrophulariaceae, Solanaceae, Verbenaceae (Kritzon, 2003).

3.2.4 Flavonoids

According to the IUPAC nomenclature, flavonoids can be classified into:

-) flavonoids, derived from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone) structure (examples: quercetin, rutin).
-) isoflavonoids, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure neoflavonoids, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure.

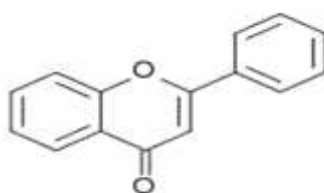


Fig. 3.5: Basic structure of Flavonoids.

Flavonoids (or bioflavonoids), also collectively known as Vitamin P and citrin, are a class of plant secondary metabolites. The research also indicated that only small amounts of flavonoids are necessary to see the medical benefits. When ingested, isoflavones are poorly absorbed because they are decomposed to more excretable fragments by a variety of oxidases as well as by bacterial fauna that thrive in our gut. Another efficient route of excretion is by way of molecular conjugation to sugar molecules. The resulting conjugates are then readily excreted from the body. Taking large dietary supplements provides no extra benefit and may pose some risks (Cristobal, 2000).

a) Rotenone (a class of isoflavones)

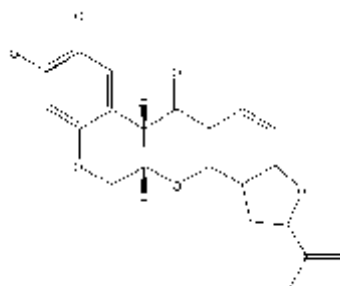


Fig. 3.6: Molecular structure of Rotenone

Rotenone is a member of a class of compounds of related molecular structure referred to as isoflavones. Rotenone is a naturally occurring chemical with insecticidal, acaricidal (mite and spider-killing) and piscicidal (fish-killing) properties. It is a selective, non-specific insecticide, used in home gardens for insect control, for lice and tick control on pets, and for fish eradications as part of water body management. It exerts its toxic action by acting as a general inhibitor of cellular respiration. However, they have been used for centuries (at least since 1649) to paralyze fish, causing them to surface (Pesticide News 2001).

Plants containing rotenone are the second most utilized as a fish poison. Rotenone is an alkaloid toxin, in a group called flavonoids and stuns fish by impairing their oxygen consumption. The plant is toxic only to cold-blooded creatures (Kritzon, 2003). Rotenone has also found significant use as a highly selective piscicide for the control or eradication of fish in streams and lakes (Islam, 2006).

The LD₅₀ for rats (the amount of the chemical lethal to one-half of experimental animals) is between 132 and 1,500 mg/kg. One factor in this wide variation may be the differences in the plants extract used. The acute oral toxicity of rotenone is moderate for mammals, but there is a wide variation between species. It is less toxic for the mouse and hamster than for the rat; the pig seems to be especially sensitive. Recent studies have shown that in rats, rotenone is more toxic for females than males. It is highly irritating to the skin in rabbits, and to the eyes. In rats and dogs exposed to rotenone in dust form, the inhalation fatal dose was uniformly smaller than the oral fatal dose. Rotenone is believed to be moderately toxic to humans with an oral lethal dose estimated from 300 to 500 mg/kg. A lowest lethal dose of 143 mg/kg has been cited in a child (Pesticide News, 2001).

Different scientists have experimented rotenone to different animals at different doses and studied the effects (Table-10).

Table: 4. Showing the doses and effect of rotenone on different animals.

S. No.	Affecting animals	Dose	Effect	References
1.	Dog	10 mg/kg/day for 6 month.	Wt. loss, change in blood chemistry.	IPCS, Op. cit. 4.
2.	Rat	1.7 mg/kg/day for 42 days.	Mammary tumors.	Extoxnet, Op. cit. 1.
3.	Hamster	120 mg/kg/day for 18 month.	No carcinogenic effect.	Extoxnet, Op. cit. 1.
4.	Guinea pig	4.5&9 mg/kg/day for unspecified period.	Foetotoxicity & failure of offspring.	Extoxnet, Op. cit. 1.
5.	Pregnant rat	5 mg/kg/day.	Young with skeletal deformities.	Extoxnet, Ibid.
6.	Human	300 to 500mg/kg	Moderately toxic.	Extoxnet, Ibid.
7.	Fish	0.02 to 0.2mg/litre	Highly toxic.	IPCS, Op. cit. 4.

[Source: Pesticides News 2001]

b) Rotenone toxicity

The toxicity of rotenone is because of its efficacy in interrupting mitochondrial electron transport which hinders the utilization of oxygen in respiratory

organisms, leading to cell death and eventually to the death of the organism if the dose is high enough. The mechanism of interrupting electron transfer is similar to other well-studied respiratory inhibitors. Because the respiratory mechanism of fish is directly linked to water through the gills, rotenone may pass directly into the blood-stream of fish, leading to death. Rotenone is much less toxic to mammals and birds because the route of ingestion is through the gut where much of the compound is broken down to less toxic components before toxic quantities can enter the blood-stream. Rotenone ingestion through inhalation results in significantly higher toxicity, as there is a more direct pathway into the blood-stream (Islam, 2006 and Clay, 2000).

Once the compound is diluted in the water column, the risk of ingestion via inhalation is insignificant because of the very low concentrations of rotenone added to the water, and the remote chance of humans, mammals, or birds aspirating treated water in huge quantities into the lungs. Because rotenone is so highly specific and highly toxic to fish, only very small quantities need to be added to a stream or a lake to kill undesired fish. The amount of rotenone administered to a water column to achieve the desired degree of toxicity to fish depends on several factors: temperature, hardness, pH, the amount of organic detritus in the stream, and the amount of sunlight that penetrates the water. Typically, the concentration of rotenone for piscicidal application ranges from 20-40 parts per billion (ppb) (Finlayson et al, 2000).

c) Toxic dose of rotenone

40 ppb (40 micrograms of rotenone per liter of water) is more adequate to kill most species of fish. The data supports that there are no harmful effects observed below a dose of 400 micrograms per kilogram. The lower value with the safety factor corresponds to a dose of 280 micrograms for a 70 kg adult (178 lb). For a lethal dose, it is estimated that an average adult would have to consume 300-500 milligrams per kg of body weight, equivalent to drinking 500,000 liters of the water or 1/5 of the water which is clearly impossible (<http://www.epa.gov/iris/subst/0344.htm>).

d) Fate of rotenone in natural water systems

Rotenone is difficult to handle in the pure state, as it is sensitive to light and oxygen, quickly decomposing to less toxic products. This feature carries over to its use in natural water systems as water solutions of rotenone exposed to sunlight decompose readily to less toxic products.

Rotenone is poorly soluble in water. Because of this hydrophobicity, rotenone absorbs to soil particles strongly, removing rotenone from solution until other chemical actions occur. The major route of photochemical oxidation is through the intermediate compound rotenolone, which is approximately an order of magnitude less toxic than rotenone. Also, in the presence of water, hydrolysis of side chain groups occurs, reducing the toxicity while also increasing the water solubility of the product, increasing the rates of desorption from soils and particulates in the water column. Retention of rotenone on streambed soils depends upon temperature (Marking, 1988).

In high altitude lakes of California with cold waters, the oxidation of rotenone with potassium permanganate occurs within 30 minutes, rendering the resulting water non-toxic to fish. (<http://extoxnet.orst.edu/pips/rotenone.htm>).

e) Proposed link between rotenone use and Parkinson's disease

There being a direct link between the development of Parkinson's disease (PD) and rotenone and other pesticides. Because mitochondrial electron transport defects have been implicated in the onset of PD, and rotenone and a variety of other compounds are electron transport inhibitors (Ling, 2003).

Rotenone seems to have little toxicity when administered orally. Feeding 75 mg/kg of rotenone for two years resulted in no observed tissue or brain abnormalities. As discussed above, oral administration results in efficient metabolism of rotenone in the gut, and so little, if any, gets into the blood-stream and into the brain. These observations argue against the oral introduction of rotenone being a risk for the development of PD, especially at piscicidal concentrations. Also important to note, only a small fraction of people that die from PD have shown abnormal electron transport chemistries in their brain tissues, the majority have normal function. This observation argues against a direct link with rotenone in PD in humans (Betarbet et al, 2000).

3.2.5 Steroids

A steroid is a terpenoid lipid characterized by its sterane core and additional functional groups. The core is a carbon structure of four fused rings: three cyclohexane rings and one cyclopentane ring. The steroids vary by the functional groups attached to these rings and the oxidation state of the rings.

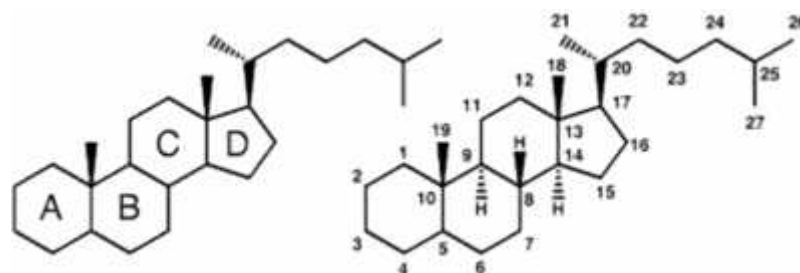


Fig. 3.7: Basic structure of Steroid.

Hundreds of distinct steroids are found in plants, animals, and fungi. All steroids are made in cells either from the sterols lanosterol (animals and fungi) or cycloartenol (plants). Both, lanosterol and cycloartenol, are derived from the cyclization of the triterpene squalene (Lanosterol biosynthesis, 2006).

3.2.6 Cardiac glycosides

Cardiac glycosides are composed of two structural features: the sugar (glycoside) and the non-sugar (aglycone - steroid) moieties.

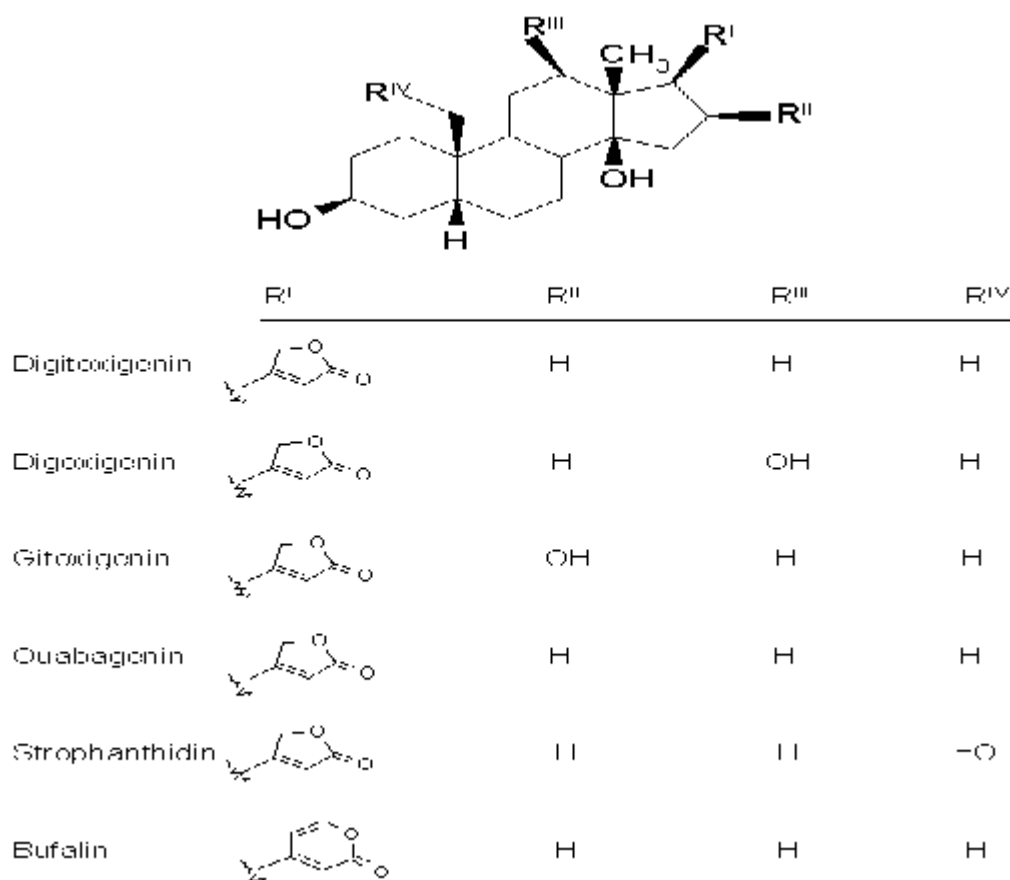


Fig. 3.8: Molecular structure of Cardiac glycosides.

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants, but also in some animals.

The cardiac glycosides are an important class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart. Plants containing cardiac steroids have been used as poisons and heart drugs at least since 1500 B.C. Throughout history these plants or their extracts have been variously used as arrow poisons, emetics, diuretics, and heart tonics. Cardiac steroids are widely used in the modern treatment of congestive heart failure and for treatment of atrial fibrillation and flutter. Yet their toxicity remains a serious problem. (["http://en.wikipedia.org/wiki/cardiacglycoside"](http://en.wikipedia.org/wiki/cardiacglycoside))

3.2.7 Terpenoid

Terpenoids can be described as modified terpenes, where methyl groups are moved or removed, or oxygen atoms added. Inversely, some authors use the term "terpenes" more broadly, to include the terpenoids.

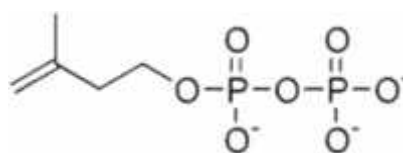


Fig. 3.9: Basic structure of terpenoid.

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Well-known terpenoids include citral, menthol, camphor, Salvinorin A in the plant *Salvia divinorum*, and the cannabinoids found in *Cannabis*.

Terpenoids (or isoprenoids), a subclass of the prenylipids (terpenes, prenylquinones, and sterols), represent the oldest group of small molecular products synthesized by plants and are probably the most widespread group of natural products.

3.2.8 Polyphenols

Phenol, also known as carboic acid, is a toxic, white with a slightly pink tinge, crystalline solid. Its chemical formula is C₆H₅OH and its structure is that of a hydroxyl group (-OH) bonded to a phenyl ring, making it an aromatic compound.



Fig. 3.10: Basic structure of Phenols.

Phenol and its vapor are corrosive to the eyes, the skin and the respiratory tract (Budavari, 1996). Repeated or prolonged skin contact with phenol may cause dermatitis, or even second and third-degree burns due to phenol's caustic and defeating properties (Lin et. al, 2006). Inhalation of phenol vapor may cause lung edema. The substance may cause harmful effects on the central nervous system and heart, resulting in dysrhythmia, seizures and coma (Warner et. al, 1985). The kidneys may be affected as well. Exposure may result in death and the effects may be delayed. Long-term or repeated exposure of the substance may have harmful effects on the liver and kidneys. The substance is a suspected carcinogen. Besides its hydrophobic effects, another mechanism for the toxicity of phenol may be the formation of phenoxy radicals (Hansch et. al, 2000).

3.3 Other Uses of Herbal Piscicides

The plant extract from different species are experimented and observed for the other treatments through different literatures as follows:

3.3.1 *Agave americana* (Ketuke)

The juice from many species of *Agave* can cause acute contact dermatitis. In human it may produce reddening and blistering lasting one to two weeks. Episodes of itching may recur up to a year thereafter, even though there is no longer a visible rash. Irritation is, in part, caused by calcium oxalate raphides (Dimmitt, 2001).

3.3.2 *Budhlea asiatica* (Bhimsenpati)

The plant has been used as an abortifacient and also in the treatment of skin complaints (Chopra, Nayar & Chopra, 1986). The juice of the plant is applied as wash to treat skin diseases. The leaf is used in worship and its extract is used as fish poison (Manandhar, 2002).

3.3.3 *Euphorbia royleana* (Siundi)

The latex of *E. royleana* has been experimented to taste the toxicity in other aquatic animals also. It has high molluscicidal and anti-choline-esterase activity against *Lymnaea acuminata* reduced the levels of 5-hydroxytryptamine (5HT) and dopamine (DA) in the nervous tissue of *L. amminata*. However, there was no significant change in the level of 5-hydroxyindole acetic acid (5 HIAA). The changes were found to be dependent on concentration of the latex extract. Similar changes were produced by both water and organic solvent extracts of the latex of *E. royleana*, thus affects all the known neurotransmission mechanisms in the snail either separately or through a complex interaction between the different neurotransmitters. This may accounts for its high toxicity to snails (Singh and Agraval, 2000).

The toxicity of *E. milii* molluscicidal latex and niclosamide (NCL) to target snails (*Biomphalaria glabrata* and *B. tenagophila*) and non-target aquatic organisms is evaluated. Latex was less toxic to oligochaeta (*Tubifx*), planktonic crustacea (*Daphnia*, *Artemia*) and considerably less toxic to Ampullariidae snails (*Pomacea*) and frog tadpoles (*Rana catesbeiana*) (Janeiro, 2000).

3.3.4 *Madhuca indica* (Mahua)

Seed of *Madhuca* yields edible oil. After the removal of the oil from seeds, the cake is locally known as Gara-Dhep. The cake is boiled in water and mixture mixed in water. 0.5 kg cake is sufficient for 10×10 feet water volume for poisoning. It is very effective poison on its application. The cake is a cheap and effective wormicide to maintain the turf, lawn and golf ground, free from earthworms.

3.3.5 *Sapium insigne* (Khirro)

Traditionally, in Nepal, the bark juice of *S. insigne* is applied on wounds to dispel worms or germs and, therefore, accelerate wound healing. The milky latex is mixed with water and given in indigestion. The tree yields an acrid, vesicant milky juice. Pammel (1911) also referred its irritant properties. Taylor et al. (1983) reported the presence of an irritant 4-deoxy-16-hydroxyphorbol esters and related compounds in this species. The juice of *S. insigne* causes irritation and smarting specially when contact with the eyes and nostrils of animals and is possible a cause of dermatitis.

3.4 Analysis of Water Quality

3.4.1 Hydrogen Ion Concentration

According to Swingle (1967), the pH of neutral water is 7. Increase in the concentrations of H^+ ions results in a lower pH value, or conversely reduction in the H^+ concentration brings about a higher pH value.

Swingle (1967), stated that waters having a pH range of 6.5-9 as recorded before day-break are most suitable for fish culture and those having pH values of more than 9.5 are unsuitable, because in pH more than 9.5, CO_2 is not available. Fish dies at about pH 11. Acid water reduces the appetite of the fish, their growth and tolerance of toxic substances.

3.4.2 Hardness

Originally, water hardness was understood to be a measure of the capacity of water to precipitate soap. Soap is precipitated chiefly by the calcium and magnesium ions present in water (Swingle, 1967).

Swingle (1967) has suggested a total hardness of 50 ppm $CaCO_3$ equivalent to be the dividing line between soft and hard waters. According to him, waters having a hardness of 15 ppm or above are satisfactory for growth of fish and do not require addition of lime, where as waters having hardness less than 12 ppm require liming for higher production of fish. Waters less than 5 ppm $CaCO_3$ equivalent cause slow growth, distress and eventual death of fish.

3.4.3 Ammonia

According to Swingle (1967), ammonia is an important nitrogenous end product of heterotrophic break down of organic matter. Certain amount of ammonia comes through the metabolically toxic excretory waste products of larger aquatic animals. Ammonia readily dissolves in water and forms ammonium hydroxide which dissociates to give ammonium (NH_4^+) and hydroxyl (OH^-) ions. Higher concentrations of ammonia are generally met in polluted water.

Aquatic autotrophs rapidly utilize ammonium ions with preference over nitrates. Toxicity of ammonia increases with pH because at higher pH, most of ammonia remains in gaseous form and toxicity decreases with low pH due to

conversion of ammonia into ammonium ion which is less toxic than gaseous form (Swingle, 1967).

3.4.4 Dissolved Oxygen

Swingle (1967) states that, dissolved oxygen is of paramount importance to all living organisms and is considered to be the lone factor which to a greater extent can reveal the nature of whole aquatic system at a glance, even when information on other chemical, physical and biological parameters is not available.

No life could exist in the aquatic system in the absence of D.O. Dissolved oxygen levels in natural and waste water depend on the physical, chemical and biochemical activities in water body. The analysis for D.O. is a key test in water pollution and waste treatment process control. Low O₂ content in water can kill fish and other organisms present in water. Organisms have specific requirement of oxygen (Swingle, 1967).

3.4.5 Biological Oxygen Demand

Biological oxygen demand is the measure of the degradable organic material present in a water sample and can be defined as amount of oxygen required by micro-organisms in stabilizing the biologically degradable organic matter under aerobic conditions (Swingle, 1967).

Swingle (1967), states that BOD is a good index of organic pollution. It helps in deciding the suitability of water for consumption. It is used in determining the relative oxygen requirements of waste waters, effluents and polluted waters. The test has its widest application in measuring waste loadings and in evaluating the BOD-removal efficiency of such treatment systems. Its application is used in carbonaceous and nitrogenous demand. In general, BOD gives a qualitative index of organic substances which are degraded quickly in a short period of time.

Table 5, explains the phytochemicals with their mechanism of action. All the chemicals have their positive and negative clinical effects except steroids and terpenoids which do not have any negative effect. Some phytochemicals shows synergetic effect with combination of other chemicals. In the pure form these phytochemicals may or may not appear more effective.

Table: 5. Major classes of phytochemicals with their mechanism of action.

S. N.	Phytochemicals	Plant source	Positive effect	Negative effect	References
1.	Alkaloids	1,2,3,4,5	<ul style="list-style-type: none"> ⌋Pharmacologic. ⌋Treat pain & flu symptoms. 	<ul style="list-style-type: none"> ⌋Anticholinergic, renal, hepatic erythrocyte toxicity. ⌋Dysphagia & dysarthria, photophobia, blurred vision, tachycardia, urinary retention, neurologic effect. ⌋Intercalate into cell wall &/ or DNA. 	Richard A. W. (2009),
2.	Tannins	3	<ul style="list-style-type: none"> ⌋Astringent, beneficial (0.15-0.2% in the diet) ⌋Potent antiviral, antibacterial, antiparasitic. ⌋Against cancer. ⌋Inhibitors of HIV replication (reverse transcriptase). 	<ul style="list-style-type: none"> ⌋Antinutritional. ⌋Anemia (at excessive dose). ⌋Bowel, kidney & stomach irritation. ⌋Liver damage & gastrointestinal pain. 	Mc G. Horald, (2004),
3.	Saponins	1,2,3,4,5	<ul style="list-style-type: none"> ⌋Pharmacologic, cosmetic & soap preparation. ⌋Used in toothpaste, soft drinks. ⌋Hydrolysis of glycosides. 	<ul style="list-style-type: none"> ⌋Toxic to cold blooded creatures. ⌋Hemolysis. 	Foerster, Hartmut, (2006);
4.	Flavonoids	2,3	<ul style="list-style-type: none"> ⌋Antiallergic, inflammatory, microbial & carcinogenic. ⌋Potent in cancer & cardiovascular disease. ⌋Antioxidant. 	<ul style="list-style-type: none"> ⌋Low toxic. 	Cushnie TPT, Lamb AJ (2005);
5.	Steroids	1,2,4,5	<ul style="list-style-type: none"> ⌋Important component of hormones. ⌋Regulate many aspects of metabolism & immune function. ⌋Maintain blood volume. ⌋Muscle & bone synthesis. ⌋Modulate fluidity of cell membrane. ⌋Antibiotic & anti-infective drugs. 		Rossier MF (2006);
6.	Cardiac glycosides	1,3,4,5	<ul style="list-style-type: none"> ⌋Treatment of cardiac failure. ⌋Increased cytoplasmic calcium concentration. 	<ul style="list-style-type: none"> ⌋Ventricular rhythm is lost (at high dose). 	"http://en.wikipedia.org/wiki/cardiac_glycoside".
7.	Terpenoids	1,4	<ul style="list-style-type: none"> ⌋Pharmaceutical function. ⌋Antibacterial, antineoplastic. ⌋Isoprenylation. 		Gershenzon J et al. (2007).
8.	Phenols	1,2,3,4,5	<ul style="list-style-type: none"> ⌋Antiseptic. ⌋Disinfectant. 	<ul style="list-style-type: none"> ⌋Highly toxic. ⌋Produce blisters in skin contact. ⌋Membrane disruption. 	Advan; Biol. Res. (2009).

Note: - 1 = *Euphorbia royleana*, 2 = *Agave americana*, 3 = *Sapium insigne*, 4 = *Budhleia asiatica*, 5 = *Madhuca indica*.

CHAPTER IV

METHODOLOGY

4.1 Study Period

To fulfill the aims and objectives the following materials and methods were used. The present investigation like field survey, collection of herbs, laboratory works was conducted from July 2008 to June 2009, covering one year.

4.2 Sources of Data Collection

The primary data was collected from observations and interviews with fishermen and local people. The information enabled to determine the different fishing implements, use of herbal poisons, poisoning strength of herbs and socio economic condition of fishermen of Banganga and Bagmati River. The secondary data was collected from reports, research papers, journals and internets.

Questionnaires were developed and distributed for collecting the information about wild use of herbal poison and its effect on fishermen. The general information about use of poison and other information were obtained by local fishermen. Direct interview method was applied for uneducated fishermen and local people. The model of questionnaire set is given in the appendix.

4.3 Study Area

The present study was carried out in western part of Kapilvastu district, Lumbini Zone and Kathmandu district of Bagmati Zone.

The Kathmandu Valley is situated from 85° 33' east to 85° 20' west and 27° 42' north to 27° 7' south. Kathmandu is the headquarters of the Central Region (Madhyamanchal). The Central Region has three zones namely, Bagmati, Narayani and Janakpur and Kathmandu is located in the Bagmati Zone. The natural vegetation is dictated by the climatic conditions and accordingly five vegetation zones have been defined for Nepal, out of which Kathmandu and its valley fall under the Deciduous Monsoon Forest Zone (altitude range of 1,200-2,100 meters). The dominant tree species under this zone comprises oak, elm, breech, maple and so forth with coniferous trees at higher elevation.

SAMPLING AREA



THE KAPILVASTU DISTRICT

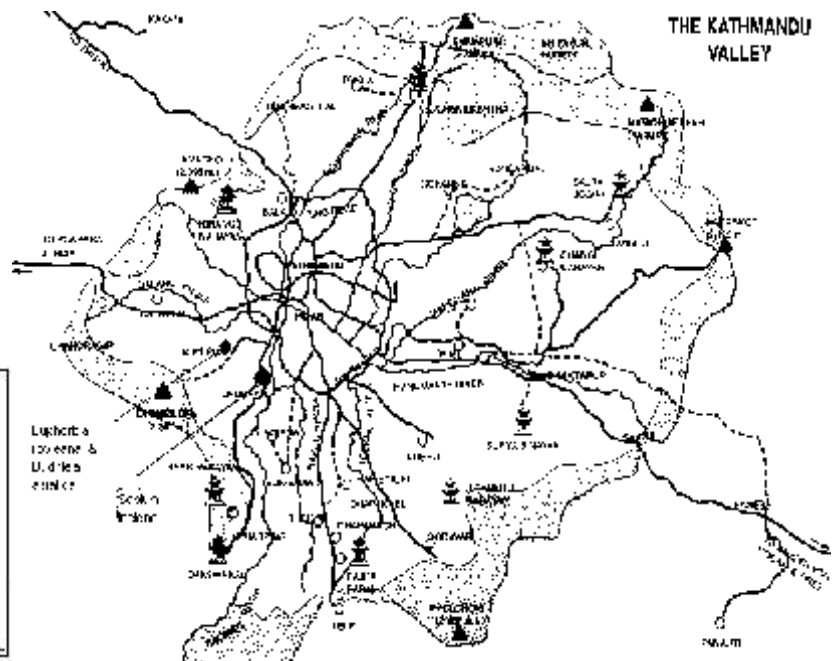


Fig. 4.1: Sampling Area; Spots showing the areas of herb collection for lab work.

The Kapilvastu district coordinates from 27° 45' N to 27° 25' S and 83° 10' E to 82° 42' west with Taulihawa as its district headquarters covers an area of 1,738 km². This belongs to the Lumbini Zone of the Western (Paschimanchal) Region of Nepal. The forest of the district stands as a natural park. The area from where the two plant samples were collected is situated at 5 km. to the southern part from the Taulihawa. The natural forest is the Sal (*Shorea robusta*) dominant forest situated 10 km. north of Taulihawa. Associated species include *Terminalia tomentosa*, *Dillenia pentagyna*, *Bauhinia purpurea*, *Ficus spp.*, *Bombax ceiba*, *Adina cordifolia*, *Accacia catechu* etc. However, the plantation forest are not restricted to a certain area, rather they are widely distributed throughout the district.

Plant Collection

For the study of toxic strength and chemical constituent present in the plant material, five different types of herbal samples were selected. The list of piscicidal plants in the study areas included their common names, scientific names, family and parts used on fish (Table-3). There were five piscicidal plants consisting of four families. The families and the number of respective genera are as follows: Agavaceae (1), Budhleiaceae (1), Euphorbiaceae (2) and Sapotaceae (1). The climate of Nepal is greatly influenced by elevation as well as by the location in subtropical latitude. Because of this, the natural vegetation of Nepal follows the pattern of climate and altitude.

Leaves of *Agave americana* were collected from Basantapur Village Development Committee, and *Madhuca indica* cake from Dharampania VDC of Kapilvastu district, Nepal, during May 2009 from the altitude range of 98 to 105 m.

Similarly, leaves of *Budhlea asiatica* and stem part of *Euphorbia royleana* were collected from the Tribhuvan University ground. The leaves of *Sapium insigne* were collected from the Chovar region near Bagmati River, Kathmandu during February 2009 from the altitude range of 1439 to 2550 m.

The plant specimens were collected from the study area along with botanical information. Local names of plants were known by interviewing the local people. The specimens were identified with the help of Botanist of Taxonomy. All the plant samples were deposited as herbarium in the Central Department of Botany. A.

americana, *B. asiatica*, *M. indica*, *S. insigne* and *E. royleana* are coded with the numbers 86, 87, 88, 89 and 98 respectively.

Collected five samples were cut into pieces and dried in aerated shade and stored at room temperature. The dried samples were powdered by traditional methods with the help of mortar locally known as Iron Musal. The fine powder were labeled in separate vessels and safely kept for experimental use.

4.4 Phytochemical Tests

All the laboratory tests and experiments were performed in NAST, Khumaltar, Lalitpur.

4.4.1 Soxhlet Extraction of Herbs

When an organic substance is to be recovered from powder form it is extracted by means of an organic solvent in which the impurities are insoluble. In actual practice the extraction from solids is often tedious and requires through contact and heating with the solvent. This is done in special apparatus, the Soxhlet Extractor. It consists of a glass cylinder having a side tube and siphon. The cylinder carries a water condenser at the top and is fitted below into the neck of the boiling flask.

The powdered material of each herb was weighed 200 gm. and placed into the thimble made of stout filter paper and the apparatus was fitted up. The methanol diluted with 50 % water is said to be 50 % methanol. The flask containing 50 % methanol solvent (MeOH 50 %) was heated on an electric heater. As the solvent boils, its vapors raised through the side tube up into the water condenser. The condensed liquid dropped on the solid in the thimble dissolves the organic substance and filtered out into the space between the thimble and the glass cylinder. As the level of liquid here raised the solution flows through the siphon back into the boiling flask. The solvent was once again vaporized, leaving behind the extracted substance in the flask. In this way, a continuous stream of pure solvent dropped on the solid material, extracts the soluble substance and returned to the flask. At the end of the operation the solvent in the boiling flask was evaporated with the help of Rotatory Vacuum Evaporator and Dessicator, leaving the organic substance behind.



Fig. 4.2: Weighing extract with the help of chemical balance.



Fig. 4.3: Soxhlet Extractor



Fig. 4.4: Drying extract with the help of Rotatory Vacuum Evaporator.



Fig. 4.5: Drying extract in a dessicator.

Soxhlet Extractor was used with advantage for the extraction of oils, fats and alkaloids from plant samples. The apparatus ensures maximum extraction with a limited quantity of the solvent.

4.4.2 Toxic strength of herbal extracts

Toxic strength of herbal extract is important to know that among five studied herbs:

- a. which one is comparatively most toxic than others and
- b. to study strength of most toxic herbs at different concentrations.

Performing such demonstrations the following procedures were conducted:

Procedures in toxic strength

The nine aquarium boxes (12"×6"×9") were set on a table. Each was filled with eight liters of unchlorinated tap water separately, among which three were selected for experiment conduction, three for positive (with chemical poison) and remaining three for negative control (without herbal/chemical poison). Following steps were involved during test of toxic strength of herbal extract.



Fig. 4.6: Testing herbal extracts on fish in a controlled condition at laboratory.

-) Each box was stocked with 10 live *Clarias batrachus* fish and left for 24 hours for acclimatization. The average weight of each fish was 4.4 gm.
-) All the aquarium fish were supplied by aquarium fish food.
-) Among nine boxes, three were selected for negative control was left as usual.
-) Three boxes selected for positive control were applied with Nuvan (0.00625 %), Dichlorvos (76 % E. C.) of Syngenta Company.
-) All fishes in positive control died within 20 to 25 minute.
-) One gram of extract in one liter of water is said to be 0.1 % concentration. The experiment is conducted in 0.05 % concentrations at 24 and 48 hours time standard.
-) The herbal extract of same concentration was mixed with glass rod in the experimental boxes to concentrate its power homogeneously.

-) After 24 and 48 hours each result were noted.
-) The remaining extract was saved for next procedure.

Fish mortalities were observed and recorded at 24 and 48 hours from stocking. Dead fish were removed immediately. A fish was considered affected by the plant toxicant when it manifests erratic swimming behavior, hyperactivity, hyperventilation and pronounced ataxia coinciding with decreased capacity to respond to visual stimuli. A fish is considered dead when it does not respond to mechanical prodding.

Lethal concentrations

The lethal dose (LD₅₀) of each of the test plant was determined by plotting concentrations of the plant against fish mortality within 24 and 48 hours after exposure to the treatment. Interpolation between two concentrations where the mortality occurred at less than and greater than 50 % was done. LD₅₀ or median lethal concentration is the concentration at which 50 % of the tested fish survived and 50 % died. It is the basis of most toxicity and tolerance tests.

4.5 Chemical Analysis of Water

The chemical parameters after and before the experiment were analyzed to know the additional cause of fish mortality with the help of fluctuation in parameters. The chemical parameters were analyzed after APHA (1998) and Trivedy & Goel (1986). The following chemical analysis was carried out:

4.5.1 Hydrogen ion Concentration (pH)

The hydrogen ion concentration is one of the most important environmental factors that affects the composition and distribution of all aquatic animals. The pH of water is negative logarithm of hydrogen ion concentration. A battery operated electrical pH meter was used to record the pH of water during the study period in the lab.

4.5.2 Dissolved Oxygen

To determine the dissolved oxygen Winkler's method was used. Water sample was collected in BOD bottle without bubbling. Then 2ml of manganous sulphate and 2ml of alkaline potassium iodide solution was added in it and shaken well. Thus, brown precipitation was obtained which was dissolved by adding 2ml of concentrated

sulphuric acid. Then, sample solution was titrated against sodium-thiosulphate solution (0.025 N). The calculation was done by using following formula.

$$\text{D.O. (mg/l.)} = \frac{(\text{ml} \times \text{N}) \text{ of Na}_2\text{S}_2\text{O}_3 \times 8 \times 1000}{V_1 - V} \quad (\text{Trivedy and Goel, 1986})$$

$$\frac{V_2 (V_1 - V)}{V_1}$$

Where, V_2 = volume of content titrated

V_1 = volume of bottle

V = volume of MnSO_4 and K

4.5.3 Total Hardness

Total hardness is the total amount of soluble salts present in the water (i.e. salts like chlorides and sulphates of calcium and magnesium). The total hardness of water was measured by EDTA titrimetric method. 50 ml of sample water was taken in the conical flask and 20 ml of ammonium buffer solution and 200 mg of Erichrome black-T indicator was added in it. Hence wine red pink color appeared. Then, it was titrated against 0.01 M of EDTA solution until a blue color appeared at end point.

It can be calculated by using following formula.

$$\text{Total Hardness (mg/l)} = \frac{\text{ml of EDTA used} \times 1000}{\text{ml of sample used}} \quad (\text{Trivedy and Goel, 1986})$$

4.5.4 Ammonia

To determine the ammonia level colorimetric method was used. 1 ml of borax buffer was added in 50 ml of water sample. 2.5 ml of 0.4 N H_2SO_4 was taken in a 100 ml conical flask and kept below the condenser. Boiling flask was placed on heater to pass the steam into the sample. Ammonia was distilled off and collected in sulphuric acid in the form of ammonium sulphate, $(\text{NH}_4)_2\text{SO}_4$. The distilled flask was removed and cooled. A blank with distilled water using the same quantity of chemicals was run. The volume of distillate was made to 50 ml and 1 ml of Nessler's reagent was added. A brown color was appeared. A standard curve between 0.05 to 2.0 mg/l of ammonia by diluting the standard ammonium solution was prepared. The measurement of ammonia was done from the standard curve.

4.5.5 Biochemical Oxygen Demand

The biochemical oxygen demand was determined by the neutralization of sample to pH around 7.0 by using NaOH. Two sets of the BOD bottles were filled with the sample. One set of the bottle was kept in the incubator at 20°C for 5 days and the DO content in another set was determined immediately. After the completion of 5 days incubation of the sample, its DO was also calculated. The calculation was done by using following formula.

$BOD, \text{mg/l} = (D_0 - D_5)$ Where, D_0 = Initial DO in the sample.

D_5 = DO after 5 days.

4.6 Phytochemical Test of Herbal Extract

The phytochemical test was performed to identify the unknown class of different compounds. The phytochemical test is necessary for the identification of toxic compounds. Performing such demonstrations the following procedures were conducted:

Procedures in Phytochemical Test

Phytochemical test was carried out according to the methods described by Sazada et al. (2009). Phytochemical analysis of the Methanol extract of *Agave americana*, *Budhlea asiatica*, *Euphorbia royleana*, *Madhuca indica* and *Sapium insigne* was which gave the test of phytochemicals like- tannins, alkaloids, steroids, phenols, terpenoids and flavonoids etc.

The following table explains the phytochemical analysis procedures of the above five plants:

Table: 6. Methodology of phytochemicals analysis of collected specimens.

S.N.	Phytochemicals	Procedure	Inference
1.	Tannins:	(200mg plant extract + 10ml distilled water + filtered) 2ml filtrate + 2ml FeCl	Blue, Black precipitate indicates the presence of Tannins and Phenols.
2.	Alkaloids:	(200mg plant material + 10ml methanol + filtered) 2ml filtrate + 1 % HCl + steam 1ml filtrate + 6 drops Dragendorff's reagent.	Creamish/ Brown/ Red/ Orange precipitate indicate the presence of alkaloids.
3.	Saponins:	0.5ml. filtrate + 5ml distilled water.	Frothing persistence indicate presence of saponins.
4.	Terpenoids	2ml filtrate + 2ml acetic anhydride + conc. H ₂ SO ₄ .	Blue, green ring indicate the presence of terpenoids.
5.	Cardiac glycosides (Keller kiliani test)	2ml filtrate + 1ml glacial acetic acid + FeCl ₃ + conc.H ₂ SO ₄ .	Green, Blue precipitate indicates the presence of cardiac glycosides.
6.	Steroids (Liebermann Burchard reaction)	(200mg plant material + 10ml Chloroform + filtered) 2ml filtrate + 2ml acetic anhydride + conc. H ₂ SO ₄ .	Blue, Green ring indicate the presence of steroids.
7.	Flavonoids	(200mg plant material + 10ml ethanol + filtered) 2ml filtrates + conc. HCl + magnesium ribbon.	Pink, Tomato, Red color indicate the presence of flavonoids, glycoside.

A '+' score was recorded in the reagent produced only a slight opaqueness; a '++' score was recorded if a definite turbidity, but no flocculation was observed and a '+++'' score was recorded if a definite heavy precipitation or flocculation was produced.

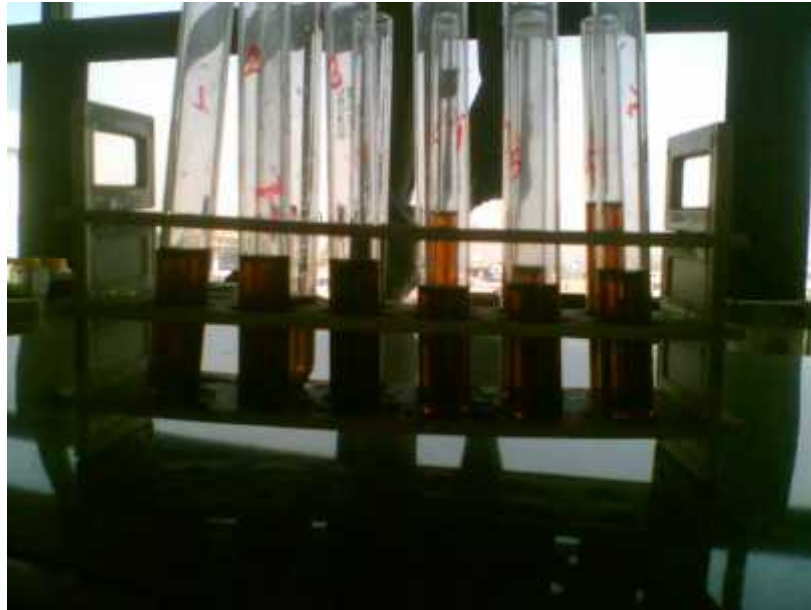


Fig. 4.7: Testing phytochemicals of herbal extract.

CHAPTER V

RESULTS AND DISCUSSION

During the study period following observations were made and the results were cited as below:

5.1 Fish Poisonous Herbs and Their Wild Use

Fish toxins or fish stupefying plants have historically been used by many hunter gatherer cultures to stun fish, so that the fish become easy to collect by hand. Some of these toxins paralyze fish, others works by reducing oxygen content in water. The process of documenting many fish toxins and their use is ongoing, with interest in potential uses from medicine, agriculture, and industry (Jeremy, 2002).

From the present study the plants, Ketuke (*Agave americana*), Bhimsenpati (*Budhlea asiatica*), Siundi (*Euphorbia royleana*), Khirro (*Sapium insigne*) and Mahua (*Madhuca indica*) were observed using as fish poison in the study areas. To kill the fishes with the help of herbs suitable places were selected on the river channel areas, small shallow pools, ditches etc. The diversion was generally about 2-3 meter wide and the slope so as to drain water completely. In this ideal channel, a stone dam is constructed by putting stones, boulders, clay, tree, trunks, branches, herbs and fern leaves. The water was deflected completely and a semi-dry fishing area was created in the down stream channel. A fish trap was set at the junction of the diversion and the main river to prevent fish from escaping. In the reduced volume of the water, the fish poisons were released. After half an hour small fishes become restless. They jump vigorously and loose their balance. They come up to the surface and were scooped up easily.

5.2 Herbal Extract

For the extraction of toxic components from plant material, methanol containing 50 % water were used. It was because; the solvents used in such extraction processes must meet critical requirements of:

- 1) being only miscible with the hydrocarbon feed stream.
- 2) having a high hydrocarbon extraction capacity and

3) having a high selectivity for aromatic hydrocarbons.

Generally speaking, the higher the capacity of the solvent the lower the selectivity. Therefore some compromise is generally made between these properties in selecting a suitable solvent for the extraction.

Other desirable characteristics of a good extraction solvent include the following: (4) low molecular weight; (5) non-corrosiveness to the equipment at operating conditions; (6) complete stability under operating conditions; (7) easy recoverability from the hydrocarbon products and (8) low cost (Eberly, 1976).

Generally, the plants with succulent leaves or stems had more extracts. The plants with the highest volume of extract obtained were *S. insigne*, *E. royleana* and *A. americana*. Although *Agave* leaf gave more extracts, it had the least piscicidal activity. Table 7 gives information about the amount of extract obtained from the collected sample. Different quantities of extract were obtained from the same amount of plant powder. The greater amount of extract was obtained from the *S. insigne* and the least amount from the *E. royleana*.

Table: 7. Amount of extract obtained from the plant samples.

S.N.	Plant material	Plant powder (gm.)	Extract (gm.)
1.	<i>Euphorbia royleana</i>	200	73.16
2.	<i>Agave americana</i>	200	56.32
3.	<i>Sapium insigne</i>	200	96.43
4.	<i>Budhleia asiatica</i>	200	45.17
5.	<i>Madhuca indica</i>	200	40.26

5.3 Ichthyotoxic Strength of Herbal Extract

The ichthyotoxic strength of all five plants was tested on fish at 500 ppm to know the most effective plant. The poisoning strength of extract was also compared with the Nuvan insecticide, and it was observed that the herbal poison is less toxic than insecticide. The time standard for the experiment was 24 hours and 48 hours. There was 100 % mortality in the positive control box while mortality was 0 % in the negative control. Among the five plants, Khirro (*S. insigne*) was found to be most poisonous and Ketuke (*A. americana*) as the least poisonous plant. Some erratic

behaviors were recorded prior to fish mortality. Such behaviors include zig-zag movement, jumping out of water, occasional jerking of tail, sprawling and rolling movement.

Results of the trial toxicity test using five piscicides on *C. batrachus* shows difference in mortalities between 24 and 48 hours of exposure to 500 ppm (Table-8). While *E. royleana* and *S. insigne* recorded 100 % mortalities within 48 hours of introduction. Test fish stocked in the higher concentrations of the test plant extracts exhibited erratic swimming behavior and rapid opercular movement. Later, the test fishes lost their balance, after which death occurred. This behavior was not evident in fishes stocked in the control and in the lower concentrations of the tested plant extracts where mortality was zero. The observation of toxic strength of different plants on fish is presented in the table 8 below:

Table: 8. Piscicidal effect of different herbs at 500 ppm. on *Clarias batrachus* (Mangur) at 24 and 48 hours time standard.

S.N.	Herbal extract	Status	Experiment time (hr.)		Positive control (%)	Negative control (%)
			Mortality (%)			
			24 hr.	48 hr.		
1.	<i>Euphorbia royleana</i>	Mortal	70	100	100	0
		Living	30	0	0	100
2.	<i>Sapium insigne</i>	Mortal	100	100	100	0
		Living	0	0	0	100
3.	<i>Agave americana</i>	Mortal	0	7	100	0
		Living	100	93	0	100
4.	<i>Budhlea asiatica</i>	Mortal	0	70	100	0
		Living	100	30	0	100
5.	<i>Madhuca indica</i>	Mortal	3	9	100	0
		Living	97	91	0	100

The effect of those plants tested on *Clarias batrachus* showed that the potency of each plant varies, which was why different percentage mortalities were recorded at different concentrations. The outcome toxicity tests proved that all the plants are poisonous to fish. Fish erratic behavior prior to death is a phenomenon associated to

impact of toxicants on fish. The result is also expressed in the figure below. With the help of figure it can be observed that the Khirro (*S. insigne*) is most poisonous showing 100 % mortality both in 24 hours and 48 hours. Siundi (*E. royleana*) also shows 100 % mortality and Ketuke (*A. americana*) is least poisonous showing 7 % mortality in 48 hours. Ketuke and Bhimsenpati (*B. asiatica*) appears non-toxic in 24 hours.

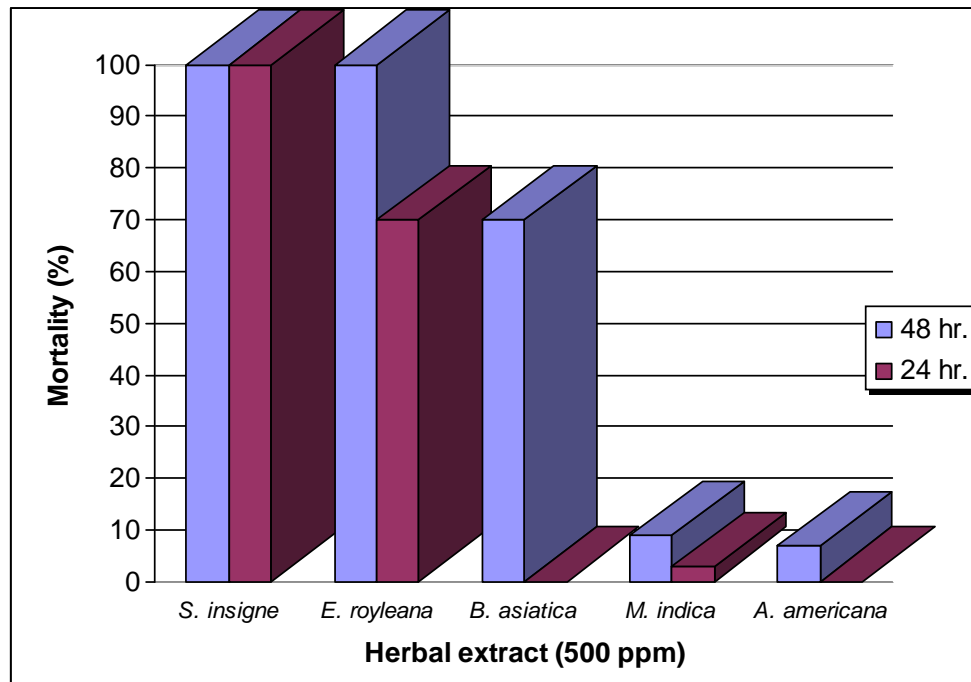


Fig. 5.1: Piscicidal strength of herbal extract on *Clarias batrachus* at 500 ppm.

The favour of some botanicals over the use of others to crop fish as reported under the results may be inferred on the basis of active ingredients contained. Fafioye and Adebisi (2001) documented *R. vinifera* pods to be more toxic than *P. biglobosa* bark on Nile Tilapia (*Oreochromis niloticus*). Similarly, Onusiriuka and Ufodike (1998) reported that *Blighia sapida* bark extract is more toxic to *C. gariepinus* than *Kigelia Africana* bark extract. The varied toxicities may be due to different active ingredients and uses of each botanical as indicated by Wang and Huffman (1991) and Alade and Irobi (1993).

Toxicity expressed as LD₅₀

LD₅₀ for fish (the amount of the chemical lethal to one-half of experimental animals) was also calculated which means that at this concentration mortality is 50 %. Based on 24 hour median lethal dose (LD₅₀), the most toxic plant to *C. batrachus* fish was *S. insigne* (65 ppm).

The toxicity to *C. batrachus* of the five plants arranged in the order of decreasing toxicity is as follows: *S. insigne* (65 ppm) > *E. royleana* (960 ppm) > *M. indica* (4100 ppm). *A. americana* and *B. asiatica* appeared no toxic for 24 hours. The results were not comparable to the results of the study conducted by Fafioye (2005) which stated that LD₅₀ value of *Agave* is 30 ppm. The difference in result may be due to the use of different solvent for extraction, fish species, age of fish and aquarium setting. Similarly, the toxicity order for 48 hours were also observed and found that *B. asiatica* (2100 ppm) is more toxic than *E. royleana* (7800 ppm).

Table: 9. LD₅₀ values of the different test plants to *Clarias batrachus* at different durations.

S.N.	Plant species	LD ₅₀ (ppm.)	
		24 hrs.	48 hrs.
1.	<i>A. americana</i>	-	7800
2.	<i>B. asiatica</i>	-	2100
3.	<i>E. royleana</i>	960	-
4.	<i>M. indica</i>	4100	-
5.	<i>S. insigne</i>	65	-

Direct comparison of the lethal dose values suggests that on increasing the time standard decreases the lethal value. The toxicity increases due to the increase of time standard.

5.4 Ichthyotoxic Strength of Khirro (*Sapium insigne*)

Among the five experimental plants Khirro (*S. insigne*) was found most poisonous plant (Fig-5.1). Therefore, this plant was selected to test its toxicity in different concentrations. The table 10 presents the mortality percent of fish by *S. insigne* in different concentrations after 24 hours time standard.

Table: 10. Piscicidal effect at different concentrations of *Sapium insigne* extract after 24 hours.

S.N.	Concentration (ppm)	Mortality (%)
1.	500	100
2.	250	100
3.	125	97
4.	62.5	3

The toxicity of *S. insigne* extract was 100 %, both at 500 ppm and 250 ppm concentration. The toxicity remained 97 % and 3 % at 125 ppm and 62.5 ppm concentrations respectively.

The herbal extract of *S. insigne* contained high ppt. of flavonoids and phenols. The higher component of flavonoids may contain rotenone which is highly toxic to aquatic animals including fish. Taylor et al (1983) also reported the presence of an irritant, 4-deoxy-16-hydroxyphorbol esters and related compounds in this species. According to them, the juice of *S. insigne* causes irritation and smarting specially when contact with the eyes and nostrils of animals and is possible a cause of dermatitis.

Toxicity experiments showed that methanol extract of *S. insigne* leaf caused significant behavioral changes in fish *C. batrachus*. Animal behavior is a neurotropically regulated phenomenon, which is mediated by neurotransmitter substances (Rao, 1999). In the present study the abnormal behavior of the fish may be accumulation of acetylcholine in the neuromotor regions. The initial increase in opercular movement can be taken as index of the stress felt by the fish exposed to plant extract. Subsequent decrease in opercular movement may be constructed a passive response to prevent excess entry of extract molecule present in the medium to minimize damage to gill epithelium (Gill et al., 1991). Similar behavioral responses were also observed in tested fishes.

High concentration of phenols could be the additional cause for fish toxicity. According to Budavari (1996), inhalation of phenol vapor may cause lung edema. The substance may cause harmful effects on the central nervous system and heart, resulting in dysrhythmia, seizures and coma (Warner, 1985). The kidneys may be

affected as well (Lin et. al, 2006). Approximately one gram is enough to cause death in human (Auschwitz, 2006).

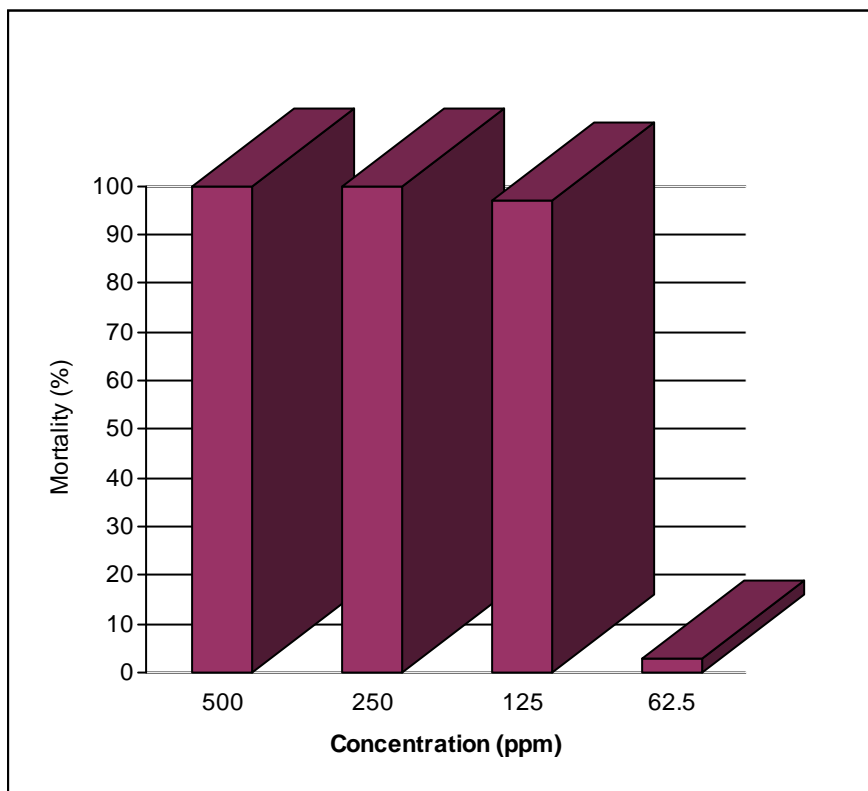


Fig. 5.2: Toxic strength of *Sapium insigne* (Khirro) extract at different concentration on *Clarias batrachus* LD₅₀ = 65 ppm.

5.5 Chemical Analysis

5.5.1 Water

The analysis of water (Table-11) shows some changes in physico-chemical parameters after the application of the poison (*S. insigne*). The changes are discussed as follows:

a) Hydrogen Ion Concentration

The pH of aquarium water remained slightly alkaline throughout the experiment. The average pH value recorded was 7.8. After the application of *S. insigne* extract at 65 ppm concentration (LD₅₀) the pH was slightly increased to 8 (Table-11). This range of pH is suitable for fish culture.

b) Dissolved Oxygen

The overall average dissolved oxygen value of the aquarium water was 13.228 ppm before the introduction of the extract which indicates the presence of good amount of oxygen in the water. After the application of *S. insigne* extract in the aquarium the oxygen level of the water is reduced to 2.818 ppm (Table-8). Further drop in DO concentrations was attributed to the oxygen consumption of the test fishes. According to Teichert-Coddington and Green (1993) cited in Egna and Boyd (1997), Nile tilapia can survive at DO concentrations of below 0.5 ppm and can live for about 6 hours. The amount of oxygen below 5 ppm is harmful and fish appeared lethargic and sluggish.

c) Total Hardness

According to Karna (1993), the natural water resources of Nepal contain more than 100 ppm of the total hardness (Lind, 1974). The total hardness of aquarium water was 70 ppm. The total hardness increased to 80 ppm after the application of *S. insigne* extract (Table-11). Water hardness above 15 ppm is satisfactory for fish growth.

d) Ammonia

The average ammonia value recorded was 0.6 ppm in fresh water of the aquarium but after the application of the *S. insigne* extract, its ammonia level was increased to 3 ppm (Table-11). The increased level of ammonia could be the causes of increased toxicity.

e) Biological Oxygen Demand

The overall average range of biological oxygen demand was recorded as 10.003 ppm. After the application of the extract poison, it is reached to 1.602 ppm (Table-11). BOD also crossed the standard limit which added the pollution in aquarium water.

The result of water quality analysis for the different parameters is presented in Table- 11.

Table: 11. Water quality analysis.

S.N.	Parameters	Test Results		Standards (Swingle, 1967)
		Before application of <i>S. insigne</i>	After application of <i>S. insigne</i>	
1.	pH	7.8	8	6.5-9
2.	Total Hardness	70 ppm	80 ppm	12-15 ppm
3.	Ammonia	0.6 ppm	3 ppm	<1.5 ppm
4.	Dissolved Oxygen	13.288 ppm	2.818 ppm	>5 ppm
5.	Biochemical Oxygen Demand	10.003 ppm	1.602 ppm	<1 ppm

5.5.2 Plant Extract

The chemical analysis of plant extract was performed to identify the unknown compound groups from the herbal extract (MeOH 50 %). The phytochemical screening was carried out on five ichthyotoxic plants of Nepal. Investigation revealed the presence of secondary metabolites in all the plant species but their concentrations varied (Table-12). Of the investigated plants, 100 % plant species contained alkaloids, tannins, saponins and phenols, 40 % showed the presence of terpenoids, 60 % flavonoids, steroids and cardiac glycosides. The leaf of all the plants and cake of *Madhuca indica* were rich in alkaloids, tannins, saponins and phenols.

On conclusion, from the present experiment it is clear that fish died due to the decreased oxygen increased ammonium, and biological oxygen demand, because these three parameters are beyond standard range. Due to the application of poison (*S. insigne*) the level of dissolved oxygen is drastically decreased and remained below the standard range. Hence, increased pH and hardness were not the cause of fish death.

Table 12, shows the presence/absence of certain class of compounds during the phytochemical analysis of herbs.

Table: 12. Phytochemical analysis of different plant species:

S.N.	Plants Name	Alkaloid	Tannin	Saponin	Flavonoid	Steroid	Cardiac	Terpenoid	Phenols
1.	<i>Euphorbia royleana</i>	++	+++	++	-	+	+	+	+++
2.	<i>Agave americana</i>	+++	+	+++	+	++	-	-	+
3.	<i>Sapium insigne</i>	+	+++	++	+++	-	++	-	+++
4.	<i>Budhleia asiatica</i>	++	+	+	-	+++	++	++	+
5.	<i>Madhuca indica</i>	+++	+	++	++	-	-	-	+

If the ppt and/or color were absent: -, slight: +, medium: ++, high: +++.

5.6 Tested Phytochemicals

The major classes of phytochemicals tested and the results were cited as follows:

5.6.1 Alkaloids

Species *A. americana* and *M. indica* contained high amount of alkaloids. The result also showed alkaloids in *E. royleana* and *B. asiatica* but trace amount in *S. insigne*.

5.6.2 Tannin

Species *S. insigne* and *E. royleana* possessed high level of tannins and low in other species of the plants.

Tannins are distributed in species throughout the plant kingdom. These are found in leaf, bud, seed, root, and stem tissues (McGee and Harold, 2004). Tannins may be employed medicinally in anti-diarrheal, hemostatic, and anti-hemorrhoidal compounds (Bate-Smith and Swain, 1962).

5.6.3 Saponin

Species *E. royleana*, *A. americana*, and *S. insigne* were rich in saponins. Considerable amount of saponins were also detected in *M. indica*. The presence of saponins and rotenone (alkaloids) in *M. indica* species have been reported also by Khanna (1996).

5.6.4 Flavonoids

Screening for the flavonoids of the plants *S. insigne* and *M. indica* gave the good positive test. Similarly, *A. americana* also contained flavonoids but in trace amount.

5.6.5 Steroids

Screening for the steroids in the plant *B. asiatica* contained steroids in high amount. Similarly, *A. americana* also gave good positive test. A trace amount of steroid found in *E. royleana* but it gave negative test in *S. insigne* and *M. indica*.

5.6.6 Cardiac glycosides

Glycosides were detected in high amount in *S. insigne* and *B. asiatica* but they were detected by low concentrations in *E. royleana*. Plants like *A. americana* and *M. indica* showed negative test for glycosides. Glycosides of plant species are known for its antimicrobial properties.

5.6.7 Terpenoids

Screening for the terpenoids of the plant *B. asiatica* contained in high amount. A trace amount of terpenoid is found in *E. royleana*. Species *A. americana*, *S. insigne* and *M. indica* showed negative test for terpenoids.

5.6.8 Polyphenols

Species *E. royleana* and *S. insigne* contained high amount of phenols. Other plants like *A. americana*, *B. asiatica* and *M. indica* showed a trace amount of phenols.

5 % Phenol is sometimes injected near a sensory nerve in order to temporarily (up to a year) stop it from transmitting impulses in some intractable cases of chronic neuropathic pain (Brown et. al, 1975).

CHAPTER VI

SUMMARY

The present study entitled “**Piscicidal Activity and Phytochemical Analysis of Effective Herbs of Nepal**” was conducted for a period of one year (July 2008 to June 2009). The study was mainly focused on herbal fish poison, its wild use and effect on fish including human health on fish consumption. This study also included the extraction of herbal poison, comparative toxicity of extract, effectiveness of more poisonous extract at different concentration on fish.

Based upon the five different herbal samples collected from different regions of Kapilvastu and Kathmandu district, the herbal poison were extracted in the NAST (Nepal Academy of Science and Technology) laboratory and its chemical analysis were done.

Among the five herbal samples namely, *Agave americana*, *Budhleia asiatica*, *Euphorbia royleana*, *Madhuca indica* and *Sapium insigne*, the *S. insigne* have been reported as most poisonous in this study. Its chemical composition and possible effects of the present chemicals on human health is explained.

The present investigation is divided into eight chapters. The first chapter deals with the introduction providing general information about the selected plant species and fishing methods with herbal poison, the justification and limitation of the study.

The objectives and literature review of the study is given in chapter two and three respectively. Chapter four deals with the details of methodology used to meet aims and objectives of the present investigation. The primary data were based on the laboratory work observations, direct field observations, interviews with fisherman and local people and with the help of photography. The secondary information was collected from different reports, journals, research papers, magazines and books. For the analysis of water quality, physico-chemical as well as biological parameters, dissolved oxygen, free carbon dioxide, total alkalinity and hardness were analyzed by using titration method. Similarly, phytochemical test was carried out according to the methods described by Sazada et al, (2009).

The fifth chapter deals with the results and discussion. The physico-chemical as well as biological parameters of aquarium water seem to be an ideal environment

for fish habitat but after the application of *S. insigne* some of the parameters especially, dissolved oxygen, biological oxygen demand and ammonia are greatly fluctuated. The toxic strength of herbal poison is not uniform. It is influenced by the present quantity of poisoning chemicals. During the present investigation, 100 % plant species contained alkaloids, tannins, saponins and phenols, 40 % showed the presence of terpenoids, 60 % flavonoids, steroids and cardiac glycosides. It was also found that saponins and rotenone (an isoflavone) are harmless for human at piscicidal concentration.

Hence, the present study provides baseline information regarding the herbal fish poisons. It gives awareness for the public about the use and disuse of the herbal poison. The herbal poisoning is harmful for aquatic fauna because it has not selective toxicity. The herbal poison could not harm the human health at piscicidal concentration. There is a need of further research to be exercised in evaluating or acting on specific claims of therapeutic benefit/harm from ingesting phytochemicals via fish poison.

CHAPTER VII

CONCLUSION

Fishes are the primitive group of cold-blooded vertebrates with gills and fins. Rivers in Nepal covers an area about 395,000 hectares. In Nepal, fish production from culture practices and capture fisheries reached 48,750 mt. in 2008/2009.

A large number of fishing implements have been devised to suit the water resources. Poisons are commonly used for fishing in different water bodies as the application of poison is the easy method of fishing. Fishing with organophosphates is not suitable for human consumption. Chlorinated hydrocarbon insecticides like endrine, dieldrin and DDT are highly toxic stable compounds, remain stored in fish tissue.

The poisons originating from plants are crushed, cut to pieces and sprinkled on the water. Fish poisoning is generally used up to about 500 ha. In running water, barriers are installed downstream where the poison is to be used in order to catch fish.

All over the globe, people use fish poisons, although very little work on fish poisonous herbs, their working mechanism and effectiveness of fishes has been done in Nepal. Addressing the numerous gaps in current information, **“Piscicidal Activity and Phytochemical Analysis of Effective Herbs of Nepal”** deals with the fish poisonous herbs, their toxic strength, phytochemical analysis and their wild use as fish toxins.

Fish stupefying plants have historically been used by many peoples to stun fish and it is safe for human consumption. Indigenous plants used for killing fish in different part of the country are Surti (*Nicotiana tobacum*), Chiuri (*Madhuca butyraceae*), Neem (*Azadirachta indica*), Simal tarul (*Manihot esculenta*), Pirrae (*Polygonum hydropiper*), Siundi (*Euphorbia royleana*), Khirro (*Sapium insigne*), Ketuke (*Agave americana*) etc. Some of these poisons paralyze the fish; others are thought to work by removing oxygen from water.

For the study of toxic strength and chemical constituent five different types of herbal samples were collected from different areas of Kathmandu and Kapilvastu district. Comparative toxicity and toxic strength at different concentrations of most toxic herb was performed. The work provides coverage of phytochemicals present in the plant samples. The plants which are rich in a wide variety of secondary

metabolites belonging to chemical classes such as alkaloids, saponins and polyphenols are generally superior in toxic property and exhibit physiological disturbances. Some alkaloids stimulate the central nervous system, while others cause paralysis. Tannins are also used as molluscicides. Some saponins are toxic to cold-blooded organisms at particular concentrations. Rotenone exerts its toxic action by acting as a general inhibitor of cellular respiration. Terpenoids play role for antibacterial, antineoplastic and other pharmaceutical functions. Polyphenols may cause harmful effects on the central nervous system and heart. Phenols are toxic group of compounds present in all of the selected plants. It is used as an antiseptic and disinfectant. It can be ingested in 3 to 4 % solution to cause neurolysis. It is a poison when taken orally and produces painful blisters in contact with skin.

The juice from many agaves can cause contact dermatitis. The leaf of *Budhleia asiatica* is used as fish poison. Extract from *Euphorbia royleana* affects the neurotransmission mechanism of the fish. Similarly the cake of *Madhuca indica* is effective piscicide. The milky latex of *Sapium insigne* causes irritation and smarting. Taylor et al. (1983) reported the presence of an irritant 4-deoxy-16-hydroxyphorbol esters and related compounds in *Sapium insigne* species. The herbal extract of *S. insigne* contained high ppt. of flavonoids and phenols. The separation and purification of pure toxic chemicals and their test on different fish species could not be undertaken due to limited time.

The chemical parameters after and before the poison application were analyzed to know the fluctuation parameters of aquarium water. The quantity of oxygen is drastically decreased below the standard level after the application of poison and the ammonia level is increased beyond standard level. The mortality percent of fishes due to the application of herbal poison compared with chemical poison is recorded at 24 and 48 hours.

CHAPTER VIII

RECOMMENDATION

» There is a need of further research to define the roles of these natural products in relation to human when ingested through fish toxins indirectly. Very great care needs to be exercised in evaluating or acting on specific claims of therapeutic benefit/ harm from ingesting phytochemicals via fish poison. Such statements require constant review; it appears that there are very limited for phytochemicals in human therapy.

» Flavonoids found present only in *Sapium insigne* and trace in *Agave americana*, they are compounds having diverse beneficial biological and antioxidant effects. Their dietary intake is quite high compared to other dietary antioxidants like vitamins C and E. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and antioxidant activities. An urgent study of piscicidally important plants is needed to establish the safety of xanthohumol or other flavonoids for use as dietary supplements since high doses of these compounds may produce adverse effects in humans.

» Isolation and identification of piscicidal substances from aquatic weeds is of great importance to reveal possible sources of environmental pollution and to find new biologically active substances for future practical use. Such work should focus in Nepal, where abundant varieties of plant resources exist.

» The separation and purification of phytochemicals along with biochemical composition of poisoned fish is also an interesting part in the study.

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APPENDIX-1

4.6.1 Materials required

Physical:

Test tube
Test tube stands
Spatula
Chemical balance
Table
Hot water bath
Glass rod
Watch glass
Pipette
Beaker
Cylinder
Conical flask
Aquarium box (12"×6"×9")
Hand net
Fish (*Clarias batrachus*)
Bucket

Chemical

Methanol
Hydrochloric acid
Dragendorff's reagent
Water
Distilled water
Acetic acid
Plant extracts
Salphuric acid
Ferric chloride
Chloroform
Ethanol
Magnesium ribbon.
Nuvan insecticide
Plant extracts

APPENDIX-2

A list of questionnaire used in interview with fisherman of the Banganga River.

1. Name: 2. Caste:
3. Age: 4. Sex:
5. District: 6. VDC:

7. How many members are in your family?

Ans: Male

Fimale

Children

8. Who earns money in your family for daily life?

9. From how many years you have been involved in this occupation?

Ans:

10. Is it your full time or part time work?

Ans:

11. Literate

Illiterate

12. Do you have your own land? If yes, how much?

Yes

No

13. What is your income per month by fishing?

Ans:

14. What do you do besides catching fish?

Ans:

15. How many members are included in fishing?

Ans:

16. How many days do you spend in fish catching in this river within a week?

Ans:

17. Which implement do you use mostly in fishing?

Ans:

18. Do you use poison for fishing?

Yes

No

19. Herbal

Chemical

20. Which season is best for fishing?

Ans:

21. How many types of herbal poisons are used for fishing?

Ans:

22. Which fish species do you capture mostly?

Ans:

23. Is there any fish disappeared from this river?

Ans:

24. Is the population of fish declining from this river? If yes, causes of declination.

Ans:

25. What is the selling rate of the fish?

Ans:

26. Is there any fish market in this area? Where?

Ans:

27. Any suggestions would you like to give for the improvement of fishery of Banganga River?

Ans: