Chapter One: Introduction

1.1. Background of the Study

Nepal is a small Himalayan country. It extends along the great Himalayan range South-Eastward from $80^0$04'E and $88^0$12' E longitude for some 800 km. It has an area of 1, 47,181sq. Km. In the South and reaches the highest peak 8848 m in the North with latitude of $26^0$22' and $30^0$27'N.

Ethnobotany deals with the study of relationship between people and plants and the documentation of the indigenous knowledge on the utilization of local plant resources by different ethnic groups or communities is one of the main objectives of ethnobotanical research. It is estimated that various communities in Nepal use approximately 1000 species of wild plants in traditional medicinal practice and majority of which await proper documentation (Rajbhandari, 2001). Thus, we should understand the value of such knowledge and conduct comprehensive ethno-medicinal studies for documentation.

Everywhere in this universe herbs are used in traditional medicine practice and Nepal is not an exception. The knowledge on herbal healing practice is prevailed since human civilization initiated. Since that unknown period of time, disease may have been originated and, simultaneously nature has also provided herbal curative remedies against those diseases.

The genus *Swertia* L. is one of the economically important genera of the family Gentianaceae. *Swertia* species distributed throughout the country between 200m-5500m. A total of 29 species have been reported from Nepal (Press *et al*. 2000; DPR 2001). However, the latest number reached to 30 as Chassot (2003) reported *Swertia barunensis* from Nepal. *Swertia* spp. occupies one of the major position in the trade of medicinal plants. Nine species have been reported under trade in different trading centers of Nepal (Barakoti, 2002) with the common name "Chiraito" except for *Swertia multicaulii*, which is called "Sarmaguru". Among these species, *Swertia chirayita* plays dominant role in trade and is considered superior in quality. *Swertia nervosa* is the main substitute of *S. chirayita* in trade. In the fiscal year 2000/01 and 2001/02 the traded amount of "Chiraito" was 337497kg. and 188415 kg. respectively (HMG/N, 2002). Most of "Charaito" is exported to India as a crude drug from where they are distributed mostly to Italy, Singapore and Afghanistan and also to United Kingdom.

Human beings are known to use plant differently in various field since the dawn of human civilization. Earlier people were able to find out the properties of plants by hit and trial methods. From the very beginning of human civilization, there has been closed relationship between people and plants. Ethnobotany, which includes beliefs, tradition, religion and culture of the particular area, simply refers to the relationship between people and plants (Rao, 1981) defined ethnobotany as a "multidisciplinary study involving the relationship between plants and aboriginal people, some knowledge of anthropology of the region, and a fair familiarity with the flora and vegetation of the region."

In this 21st century it has been noticed that people are much more interested in ethnomedicine than in synthetic drugs. The reasons behind are the products of plants are natural, easily available, have no side effect and the effects are long lasting.

However, the country is rich in floral diversity and share 2.7 % of the total flowering plant of the world. Out of 5866 flowering plants recorded in Nepal, 690 species are considered having medicinal properties (Malla and Shakya 1984). Among these, 510 are wild species, 120 are cultivated species and 60 are exotic (Sharma *et al.* 2004).

Allopathic treatment is spreading with rapid progress worldwide. However, the market of the herbal medicine in the developed country is increasing at a faster rate than other pharmaceutical products. Plants are very important sources of medicine due to groups of chemical compounds that may have pharmaceutical actions.

Natural population of plant shows intricate pattern of variation (Brigg and Walters, 1997). Intra and interpopulational variation in nature are nearly of quantitative rather than discontinuous kind (Falconer, 1981). Variations in the phenotypic traits may be due to environmental or genetic control to which it is exposed (Joshi and Joshi, 1998). Significant variation between progenies derived from a single maternal parent have been taken to indicate that a significant heritable component exist for measured characters (Jones, 1971). Variation in plants may be within and among populations.

The genus *Swertia* L. is the member of family Gentianaceae. It is morphologically diverse but taxonomically distinct group of about 150 annual, biannual or perennial herbaceous species (Chassot, 2003). The genus is cosmpopolitan in its distribution. However, most of the species occur in temperate regions of Northern Hemisphere. Earlier, Hara (1982) reported 27 species from Nepal. Latest Press *et al.* (2000) and DPR (2001)

enlisted 28 and 29 species respectively, but the latest number reached to 30 species including *Swertia barunnsis* as reported by Chassot (2003).

1.2 Taxonomic Description

Swertia chirayita is one of the medicinally important herbs growing naturally in Himalaya, between 1200 and 3000m. from Kashmir to Bhutan and in the Khasia-hills of India (Watt 1972; Anonyms, 1976).

Swertia chirayita (Roxb.ex. Fleming) H. Karst

Common names: Chiraito, Tite (Nep.): Khalu (Npbh.); Tikta, Gyatin (Am); Kiratotikta (Sons); Chireta (Eng.)

Description: An erect annual herb, 60-125 cm. tall stem robust, branching, cylindrical below, 4-angles upwards, containing a large pith. Leaves; broadly lanceolate, 5-nerved, subsessile. Flowers; greenish yellow, tinged with purple, in large panicles. Capsules; egg-shaped, many sided, sharp pointed. Seeds; smooth, many angled.

Conservation status: Vulnerable (IUCN category)

Parts used: Whole plant

Uses: Plant is bitter, cooling, laxative,anthelmintic, antipyratic, antiperiodic, galactogogue; cures thirst, biliousness, leucoderma, inflammations, burning sensations, pain in body, urinary discharges, ulcers, asthma, bronchitis, leucorrhea, piles, bad test in mouth, good for vomiting in pregnancy, astringent, tonic, stomachic, improve eye- sight, scabies, skin disease, chronic fever, the stem in combination with other drug, is prescribed in treatment of scorpion sting, but it is not in anantidote to scorpion venom. It is antimalarial and antidiorheatic also.

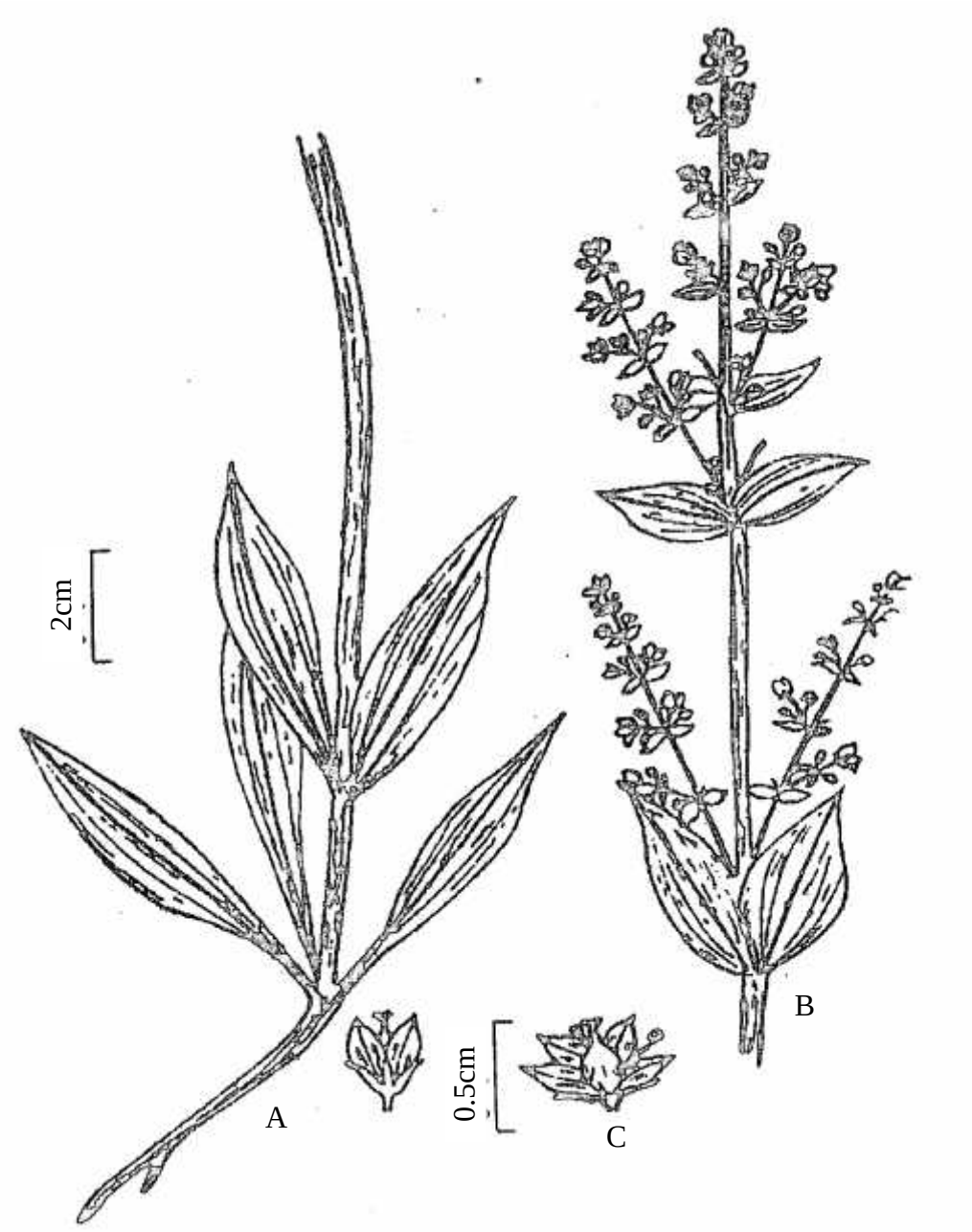


Fig. 1: *Swertia chirayita*
A. leafy branch, B. flowering twig, C. L.S. of flower

Short description of other species of *Swertia* (Family-Gentianaceae) are described below:

Swertia angustifolia Bush-Ham.ex. D. Don var.

Common name: It is commonly called as Chiraito, Tite (Nep.); Khalu (Npbh.); Ngu tig (Am); Chiretta (Eng.).

Description: An erect herb to 90cm tall leaves lanceolate, narrow at the base, 1-3-nerved. Flowers; white or pale blue in panicles, capsule; ovates.

Distribution: WCE; at 600-2600m.

Himalaya (Kashmir to Bhutan), N. India, Myanmar, S. China *Swertia angustifolia* var. pulchella Burkill: WCE, alt 2000m. Himalaya (Utter pradesh to Bhutan), India, Myanmar, China. *S. angustifolia* var. wallichiane Burkill; CE; alt 600m Himalaya (Nepal Sikim).

Part(s) used: Whole plant.

Uses- The plant is used as blood purifier and febrifuge.

Swertia ciliata (D. Don ex. G. Don) B.L. Burtt.

Common name(s): Charaito, Tite (Nep.) Khalu (Npbh.); Chiretta (Eng.)

Description: An annual herb, 90cm. tall. Stems erect or 4-angled, Leaves; oblong or lanceolate, base- narrowed. Flowers; purple or dark red.

Distribution: WCE; alt 2800-4000m.

Afghanistan, Himalayas (Kashmir to Sikkin)

Parts used: Whole plant

Uses: Used as substitute for *Swertia chirayita*

S. nervosa (G. Don) Clarke

Common name: Chiraito,Tite (Nep),Khalu(Npbh),Chireta).

Description: annual herb 60cm. stems; quadrangular, winged, leaves; elliptic to lanceolate, flower; tetramerons, corolla tube 1-2 mm; lobes green or whitish with purple markings, elliptin-ovate, ovary; ovoid, capsule; ovoid.

Distribution: 700-3000m

Uses: Used as substitute for *S. chirayita.*

1.3 Chemistry

Because of reputed medicinal value, more and more attention of phytochemists have been focused for chemical investigation of *Swertia* species. A large number of

structurally dissimilar compounds such as xanthones, flavonoid, steroids, alkaloids, terpenoids, coumarins, bicyclic hydrocarbon etc. have been reported from *Swertia* species.

1.4 Environmental Justice

Environmental justice means the fair treatments of people of all races, cultures and income levels with respect to the development, implementation and enforcement of environmental laws, regulations and polices.

The environmental programs are likely to be successful when they have the dual objectives of protecting and optimizing ecological benefits as well as improving the people's livelihood including poor, disadvantaged and landless people. It's the poorest and marginalized people that have least access to natural resources but suffer the most by environmental degradation. Therefore, management of environment would be sustainable ecologically, economically and socially only if the overall management programmes can be made suitable to the local communities to substantially fulfill their livelihood.

The first national people of color environmental leadership summit field on October 24-27, 1991 in Washington D.C. drafted and adopted 17 principles of EJ (Appendix I).

1.5. Benefit Sharing

Swertia chirayita, one of the medicinally important herb, is considered as one of the cash crop in Nepal Himalaya (daniggehis, 1996).

Although there is huge trading of Chiraita , there is not studied about the benefit sharing on trading of Chiraita. Generally grass root level people and farmers, are less benefited than trader. This scenario creates conflict between farmers and traders. So benefit sharing should be equitable for collectors, middleman and traders.

1.6 Justification

Though Nepal is rich in herbal plants having peculiar medicinal properties, there is no limitation of harvesting these herbs. Due to these factors, most of the species are being threatened and gradually disappearing. Present study has been carried out in order to find out the uses of "Chiraito" through indigenous knowledge, its preservation through different means of conservation and to make people aware of its importance. This study will be helpful to record status of uses of "Chiraito" and benefit sharing among stakeholder in relation to social equity in that area.

Genetic erosion is the most agonizing aspects of conservation biology and Chirayito can't be out of it. Raising of agricultural crops, lack of proper knowledge about Chiraita, and other unsustainable activities led to the over-exploitation from the forest area.

As *Swertia* is being taken as an traditional drug, it is necessary to study about its chemistry. Therefore, its phytochemical screening was carried out to explore naturally occurring compounds with in these species.

In prehistoric time, the traditional medicinal practices have been used medicinal plants and plant products as crude drug known as Jaributi to cure different disease without knowing its actual bioactive constituents and its chemistry. So it is necessary to carry out scientific research on the medicinal plants so as to find out its biologically active constituents with its chemical structure.

1.7. Objectives

The main objectives of present investigation include:

- To study ethnomedicinal uses of *Swertia chirayita* in Pokharathok, Arghakhanchi.
- To study the status of benefit sharing in trading of *Swertia chirayita* in local community.
- To analyse problems and prospects of conservation of *Swertia chirayita*.
- To carryout phytochemical screening of different species of *Swertia*.
- To isolate and characterize of chemical constituents of *Swertia chirayita*.

1.8. Limitations

A limited period of time does not allow undertaking progeny test though they are the confirmatory test of various studies. Another limitation of the study is conflict prevailing within our country. Non-availability of chemicals and proper laboratory is also the limitation of the study.

Chapter Two: Literature Review

2.1 Previous works on ethnobotany

A lot of works concerning ethnobotany and medicinal plants were done outside the country as well as inside the country. An attempt has been made to summarize most of the work done inside the country. Relevant literatures on the ethnobotanical as well as environmental justice within the country and abroad have been reviewed as follows:

In Nepal, the study of ethnobotany probably started with the work of Banerji (1955) who studied edible and medicinal plants from east Nepal.

Similarly, the work of Singh (1960), Manandhar (1974), Dobremez (1976), Sacherer (1979) are the earlier work on the ethnobotany of Nepal.

Manandhar (1980 a) described the medicinal plants of Nepal Himalaya along with their active constituents. Similarly, Manandhar (1980 b) enumerated some less known medicinal plants of Rasuwa district.

Malla and Shakya (1984-1985) mentioned a list of 630 medicinal plants distributed at different zones as tropical, sub-tropical, temperate, subalpine and alpine zone.

Amatya (1986) discussed the ethnomedicinal uses of tannin bearing medicinal plants of Bara district. Sixteen plants have been enlisted along with their ethnomedicinal uses and percentage of tannin present.

Bhattarai (1987) described the traditional pharmaceutical practice in central Nepal with different forms of drugs like solid dosages form, liquid dosages form, ointment, pills and so on. He also mentioned the knowledge of traditional healers, source of preparation, administration and storage of drugs.

Bhattarai (1989) studied villagers perception on forest and forest and forestry development in Chisapani village, Illam district in which he generalized that local people are positive towards afforestraiton and other forest conservation activities.

Chaudhary (1989) presented information about the medicinal plants and traditional medicinal practice in Nepalese context. The report emphasizes the scope of medicinal plants and its importance, which fills up the gap of konweldge of the existing plant wealth and uplifts the economy of the country.

Balic (1991) studied the economic value of traditional medicine from tropical rain forests, including the non-destructive and destructive use of plants their uses and method of collection.

Tremendous works were done in the year of 1991, 1993 and onwards. Bhattarai (1992 a) described the folk herbal remedies of Sindhupalchowk district, Central Nepal, with 63 plants species along with their therapeutic doses and medicinal application.

Bhattarai (1992 c) described medicinal ethnobotany in Karnali zone and he described information on 80 empirically prescriptions involving 62 plants speices including one species of fungus, one species of fern, five species of gymnosperms and 55 species of angiosperms. Bhattarai (1992 d) collected the ethnobotanical information on veterinary medicine for the first time in the country. He gave the information of 58 plants species with their definite uses for the treatment of various ailments like gastritis, fever, bone fracture, minor injuries, etc. Shrestha (1992) described 51 plants species used as medicine belonging to 49 genre and 31 families used by the people of Lele for various diseases.

Cole (1992) wrote in empowerment laws that the dumping of polluting sites are products of a political process which has historically excluded poor people and in which poor people remain grossly under represented.

Mandar and Chaudhary (1992) studied medicinal pants and their traditional use by tribal people of Saptari district. altogether, 64 plants species were reported to treat 44 different diseases with their local name parts used, forms of medication and method of use.

Kharel (1993) studied women participation in community forestry in which he explained that most committees do not include women as members due to various committee level, physical, social, political and administrative factors. Even when women are symbolically included, decision often fail to recognize women's needs and constrains.

Pant (1996) studied women participation in user groups and user committee. This study conducted in Lamachaur and Hemja VDCs of Kaski district, explores women's role in decisions related to protection, plantation, harvesting and benefit sharing in community forestry. It also assesses the training need for women.

Poudyal (1997) studied women's participation in forest management through community forestry in which he focused on various aspects of women's participation in the community forest groups in Puranchaur VDC of Kaski district. In both groups women's participation in decision making is found to be very poor.

Poudel (1998) studied the impact of community forestry program on the less privileged people of Dolkha district, in which he found that the less privileged people were getting less benefit from community forest than other class of users.

Mahato (1998) discussed the medicinal use of 19 important common plant used by the tribal people, especially the Magars of Jhadewa VDC in Palpa district.

Chhetri (1999) recorded 90 species of medicinal and aromatic plants (MAP) belonging to 81 genera 51 families have been recorded from Manang district and the highest number of species were recorded from Manang district and the highest number of species were recorded from the high altitude area (3000-5000m).

Joshi and Joshi (2000) described the genetic heritage of medicinal and aromatic plants of Nepal Himalaya. They described the plants up to their plant parts, used, important biochemical constituents and their taxonomic description.

Oli *et al* (2000) studied the local knowledge on plant use among major ethnic groups living in the Churiya of eastern Nepal and documented 82 species of medicinal plant used of treatment of 50 different disease and 76 wild edible plants.

Pokharel (2000) studied indigenous forest management practices in some community forest of Nepal in which he explained that in indigenous forest management, equitable distribution of product was essential for smooth management of forest. Unequal benefit sharing invited problems and conflicts.

Bhusal (2001) studied participation and equality in community forest users groups of Tanahun, Nepal in which he showed that there was a relatively lower participation of women, Dalit and poor in the community forest user groups where as the participation was high in the output activities i.e. benefit sharing.

Sherpa (2001) studied the high altitude ethnobontany of Walung people of the Walangchung Gola. Altogether 69 wild plant species were reported along with their short description and idea uses.

Bhattarai (2002) discussed the use of 72 plants species being used by the Bhotiya and Sherpa communities around Makalu Barun Naitonal Park and buffer zone for medicinal purpose.

Panthi and Chaudhary (2002) reported the plants of ethnobotanical importance of Arghakyhanchi district, west Nepal. Altogether 394 plant species were reported.

Chaudhary and Jha (2003) have summarized important issues of environmental justice and social equity in context of Nepal, which are (1) over population, poverty and pollution (2) environmental cartizon (3) food security and sustainable livelihood (4)

unemployment and social injustice (5) inadequate sanitation (5) dumping wastes (7) industrial effluent and toxicity (8) tonic schools (9) Air pollution (10) water pollution (11) Climatic justice (12) Eco-tourism and equity (13) lack of representation in decision making (14) corruption and (15) transboundary issue weak enforcement of legislation.

Ghimire (2003) stated that there are several discriminatory and injust practices in both urban and rural areas of Nepal, varying from disproportionate sharing of ecological benefits and hazards in society to the unequal access to resources, healthy environment, decision making information and other civil rights.

Northridge *et al*. (2003) studied environmental equity and health, in which they invoke a population health perspective to assess the distribution of environmental hazards according to race/ethnicity, social class, age, gender and sexuality and the implications of these hazards for health. The unequal burden of environmental hazards by Africa, American, Native American, Latin and Asian American/pacific Islander communities and their relationship to well documented racial ethnic disparities in health have not been critically examined across all population groups, regions and ages. The determination of existing environmental inequalities also require critical research attention.

Pakia and Cook (2004) studied the ethnobotany of Midzichends tribes of the coastal forest area in Kenya in which they described some medicinal plant species used for medicinal purposes are known to posses therapeutic characteristic while other medicinal plants are used only on the basis of mythical beliefs with the society. However, much of the traditional knowledge on medicinal plants used by the Midzichenda has not been tested pharmacologically.

A book published by IUCN and HMG-Ministry of Forestnad Soil conservation 2000, mentioned some medicinal plants with their scientific name, family name, common name, major documentation, conservation status (HMG/N), IUCN-category and CITES-category with traditional uses.

2.2 Previous works on phytochemical screening

Gao *et al* (2003) studied the determination of effective constituents in 11species of *Swertia* and related plants by HPLC. This paper deals with quantitative determination of three iridoids-Swertiamarin, gentiopicroside, sweroside and xanthone-Swertianolin in eleven *Swertia* species and related plants by means of HPLC.

Bhatia *et al* (2003) studied morphological and chemotype of certain Indian species of *Swertia*. *Swertia chirayita* Buh. Ham. is a source of an important Ayurvedic drug and the plant is scarce in wild to fulfill its commercial demand. As a result, other related species are substituted in the trade for Chiraita. The present study of five species of *Swertia* viz. *S. angustifolia* Buich-Ham. ex. D.Dom, *S. lurida* Royle ex. D.Don, *S nervosa* (Wall.ex. G-Don) CB Clarke, *S. alternifolia* Royle and *S. cuneata* wall. is in continuation to the earlier study and describes comparative evaluation of morphological characters and Thin layer chromatography (T.L.C.) finger-print profile for xanthone and Secoridoid bitters.

Kumar *et al* (2003). *Swertia chirayita* (Roxb. ex. Flem.) H. Karst mediated modulation of interleukin-1β interleukin- 6, interleukin-10, intmerferon-r and tumor necrosis factor-α in anthritic mice. This study will help in our understanding of the mechanism of anti-inflamatory action of *Swertia chirayita* in the light pro- inflammatory and anti-inflamatory cytokine balance.

Sakagami *et al* (2003) studied the development of a method to reduce microbial numbers in powdered crude drug by a copper-alcohol treatment. Its results suggest that this treatment method would be very useful to reduce the number of micro organisms in powered crude drugs.

Tan Gui-Shan *et al* (2003) studied the active constituents of *Swertia davidi* Franch. Chemical components were isolated by column chromatography and their structures were established mainly by spectroscopic means; UV, IR, NMR, 2D-NMR, MS. μ-Bondapak C_{18} was used as the stationary phase and water-methanol-isopropanol-tetrahydrofuran (65:30:5:1) as the mobile phase. Analytical results were given. The similarities and diversities of these plants were compared and discussed.

Yang wei-Xia *et al* (2003) summarized the present circumastances of studying the chemical components of the medicinal plants of the gentianaceae as well as the general

methods of extracting it. The same time, the iridois and secoiridoids from this family founded in recent years are summed up.

Hajimehdipour *et al,* H.Y. Amanzadeh, S.E Sadat Ebrahimi and V. Mazaffarina (2003).Studied three tetraoxygenated xanthone from *Swertia longfolia*. Three xanthones were isolated from petroleum ether extract of the aerial parts of the herbs, *Swertia longfolia*, an endemic plnat of flora Irinica. The structures were confirmed by various spectroscopic methods; UV, IR, ^{1}H-NMR, ^{13}CNMR and MS) as swerchirin, swertiaperenine and gentiacauleine.

Shi Gao-feng *et al* (2004) studied on chemical constituents of essential oil from *S. chirayita*. The chemical constituents were isolated and were identified by capillary GC-MS method. Sixty-three compounds from the oil were identified and using known alkane standard.

Zeng Guang-Yao *et al* (2004) studied water soluble chemical constituents of *S. Davidi* Franch. Column chromatographies on silica gel, Sephadex Lh-20 and Diazon-201 *et al*. were used to isolate and purify the chemical components. Their structures were identified by UV, IR, MS, NMR and 2D-NMR.

Yang *et al* (2004) studied the level of seven important phytochemical constituents of *S. franchetiana* and statistically compared, using materials collected form sites ranging from 2200-3960 m. in altitude. Swertiamarin was the most abundant in all samples, then mangiferin, Olenolic acid and the other three Xanthones. Throughout the distributional range of this species, no altitudinal trend was detected for other constituents except 1, 8 dihydroxy-3, 7 dimethoxyxanthone, which shows a negative correlation with altitude. However, the concentration of 1,8 dihydroxy-3, 7 dimethoxyxanthone and mangiferin showed a significantly latitudinally and longitudinal correlation.

Vishwakarma *et al* (2004) studied a sensitive HPTLC method for estimation of Swertiamarin in *Enicostema littorale* Blume, *Swertia chirayita* (Wall) Clarke, and in formulations containing *E. littorale*. the method was used for estimation of the Swertiamarin content of whole plants of *E. littorale* and *S. chirayita.* The method is suitable for quantitifcaiton for swertiamarin in samples containing amounts ranging form 0.15 to 7.7 % (W/W).

Ji Lan-Ju *et al* (2004) studied on the chromatographic finger print of *Swertia franchetiana* by HPLC. The chromatogrpahic fingerprint of *S. franchetiana*. H. Smith has

been set up by HPLC diode-array detection method for ten batches herbs collected in different district of Quighai-Tibet platewe. The analysis was performed on a VP-ODS C_{18} (5 μm, 150 mm x 4.6 mm) eluted with methanol and 0.02 % aquous phosphoric acid as mobile phase and with UV detection at 254 rim. A general fingerprint acquaintance of main constituents in *S. franchetiana* can be obtained by HPLC method which provides scientific indexes for quality of the *S. franchetiana*.

Ji Lan-Ju *et al* (2004) determined active constituents of 15 species of *Swertia* by HPLC. This appear deals with quantative determination of three iridoid glycosides; swertiamarin, gentiopicroside and amarogentin. The similarities and diversities of these plants were compared and discussed.

Kumar *et al* (2004) studied the correlation of cytokines and mobility in mice with anthritis and during therapy with *S. chirayita*. They studied a correlation between pro-inflamatory cytokines and mobility in anthritic mice after treatment with *S. chirayita* plant extract.

Carnat *et al* (2005) studied the influence of drying mode on iridoid bitter constituent level in gentian root. Root samples of wild gentian were harvested from six localities (attitude 970-1350m). In all the samples, levels of iridoid bitter constituents and of xanthone coloured compounds were determined by HPLC.

Wang *et al* (2005) reported two new iridoid glyosides from the *Swertia franchetiana*. Two new iridoid glycosides designated as senburiside III (2) and senburiside IV (3) together with one known iridoid glycoside senburiside I (1) and three known secoiridoid glycosides Swertiamarin (4), gentiopicroside (5) and sweroside (6) were isolated from the whole plant of *S. franchetiana*. The structure of the two new compounds were eluciated by spectroscopic method.

Ma Yu *et al* (2005) determined dynamic accumulation of medicinally bioactive components of *Swertia mussotii* Franch. in different growth period. The content of six species medicinal bioactive components *S. musotti* Franch in different growth periods were determined by reversed-phase high performance liquid chromatography. Result showed that the whole plant should be harvested during September and October.

Piatizak *et al* (2005) studied the secoiridoid accumulation (expressed as a sum of gentiopicroside, sweroside and swertiamarin) in shoot after 21 days of culture reached about 303 mg/l.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Ethnomedicinal study of *Swertia chirayita*

This study has been carried out in order to find out the traditional ethnomedicinal knowledge about *S. chirayita*. It also aims to assess the attitude about benefit sharing. The methodology employed to fulfill the objectives of the present study is described below under different topics:

3.1.1 Location of the Study Area

For the ethnomedicinal study of *Swertia chirayita* Pokharathok V.D.C,Argakhanchi was selected. Pokharathok VDC has covered the area of 22 sq. km. (Approx) and lies in Mahabharat range with attitude of 1830 m. It is situated between the latitude of $27^0$50' to $27^0$54' north and longitude of $83^0$16' to $83^0$18' east.

For convenience, the study area has been conducted in 9 wards. The study area is surrounded by Satyawati, Patauti, Juthapauwa, Panena and Khidim (Map. Pokharathok VDC).

3.1.2 Collection of Information

The primary infomration regarding the uses, used plant parts, values of plants and status of social equity towards the using pattern of *S. chirayita* in the study area. The field work comprises two approaches i.e. survey technique and inventory technique (Martin, 1995; Cunningham, 2001). The survey technique included individual and in depth interviews and focus group discussion among the local plant users, school teachers, villagers. The inventory technique comprised the collection of *S. chirayita* from study area and identification of their local names, parts used, purpose of the use etc. with the participation of knowledgeable key interviewers as well as with local people.

3.1.3 Field Visit and Household Survey

The field was visited 2 times during the month of August-September 2005 and Jan.-Feb. 2006. The household survey was conducted on the basis of random sampling method. All household of study area were categorized into two classes i.e. privileged and deprived.

Interview was conducted with key informants like traditional healers (Dhami/Jhankri, Baidhya) and local people. A total 40 informants was selected for interviews. The data were taken by making questionnaire.

The structure of questions have been given in appendix II.

A standard questionnaire was prepared for interview with local healers, middle man and traders. Survey was also conducted to assess attitude of environmental justice in terms of benefit sharing among stakeholders.

3.1.4 Method of Interview

The information about traditional knowledge and economic uses of plant and their impact on environment was obtained trough interviews with local people.

All the data were cross-checked and rationalized with the villagers to make it more informative.

3.1.5 Secondary Data Collection

The population data were collected form statistical year book of Nepal 2005,population census of Nepal 2001 and map was collected form Napi-Bibhagh, New Baneshwar. All necessary literatures were collected from central library, Kirtipur and library of DPR,IUCN and ICIMOD.

3.2 Phytochemical screening

3.2.1 Plant Materials

Different species of *Swertia* were collected from different localities viz. *S. ciliata* (D.Don ex. G. Don) B.L. Burtt from Yetikharka, Manang.

Swertia nervosa (G.Don) Clarke from Chhahara, Palpa,

Swertia angustifolia Buch-Ham-ex. D.Don var from Phulchoki, Kathmandu and four natural population sites of *Swertia chirayita* from different geographical regions of Nepal were selected for investigation. These sites include Sova Pokhari in Sankhuwasava (*S. chirayita* A.), Pokharathok in Arghakhanchi (*S. chirayita*, B), Mai Maghuwa in Illam (*S. chirayita*, C), and Solma in Terhathum (*S. chirayita*, D.) And these were identified in Central Department of Botany Tribhuvan University, Nepal by comparing the collected specimens with the authenticated herbarium sample presented at TUCH.

3.2.2 Extraction

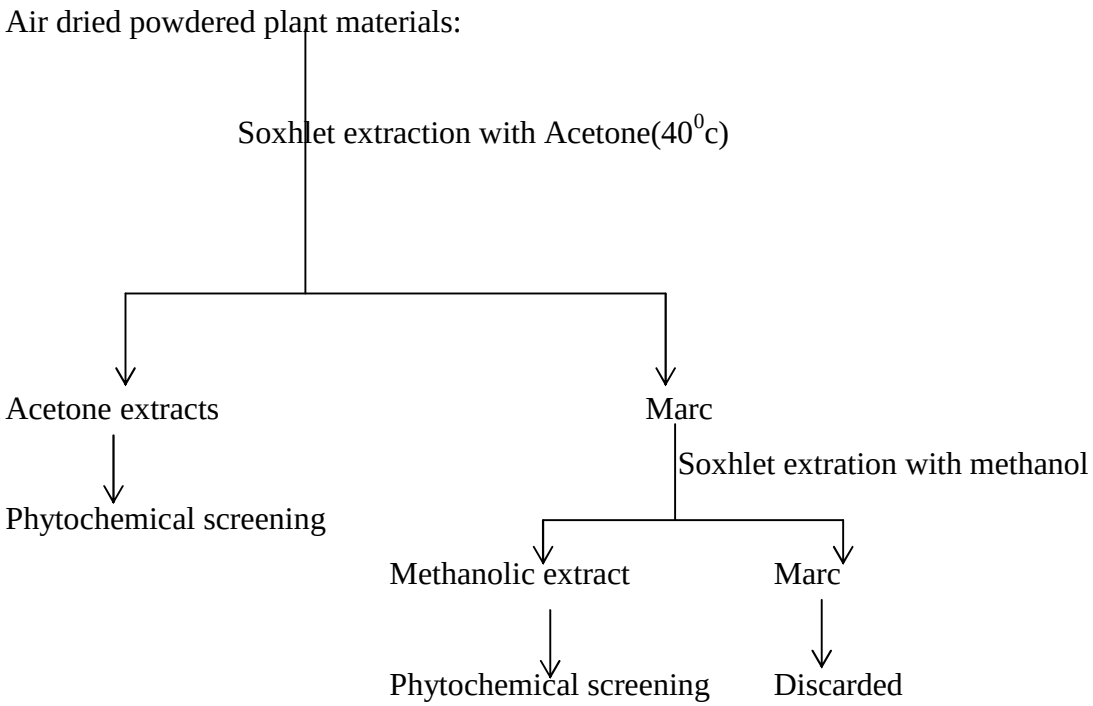
Air dried powdered plant material from different samples, *S. ciliata* (70.1gm), *S. nervosa* (35gm), *S. angustifolia* (18.65gm.), *S. chirayita*; sample A (35.3gm). Sample B (30gm), sample C (35gm) and Sample D (40gm) were subjected to extract separately with Acetone (500ml) using Soxhlet aparatus each for ten hours. Each acetone extracts was concentrated using simple distillation apparatus over water bath.

After the complete extraction of sample with solvent Acetone, Methanol was used on the marc for further extraction. Then each methanolic extract was concentrated using Rota vapours.

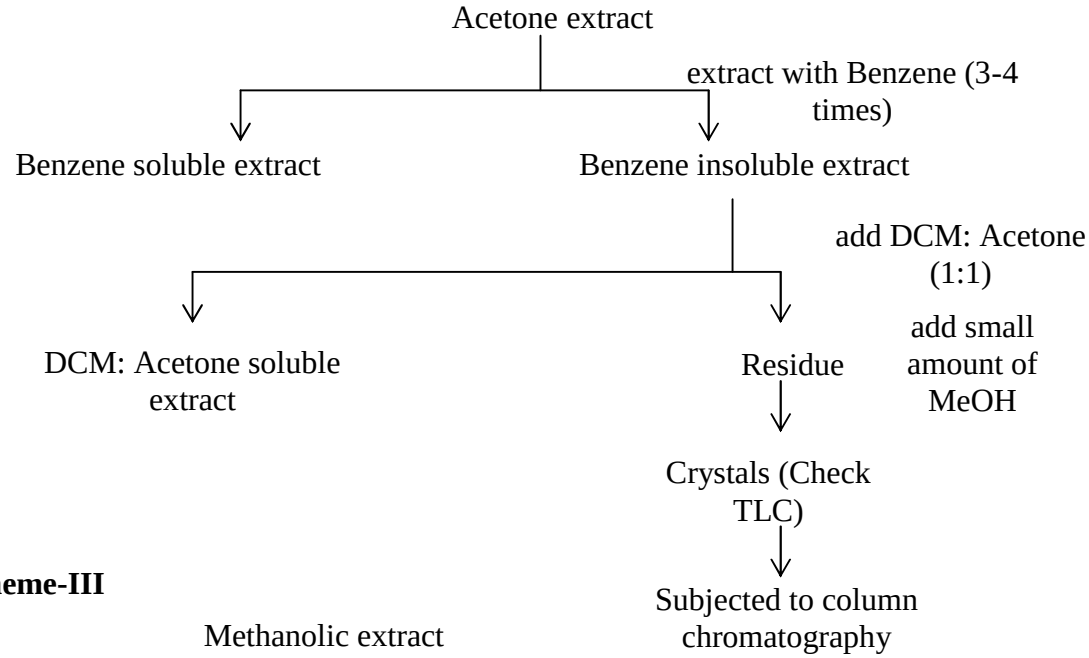
$$\text{Percent of chemical extract} = \frac{Wt.\ of\ \ extract}{Wt.\ of\ \ plant\ material\ used} \times 100$$

The process of extraction is given in the following flow chart (Scheme I, II, III).

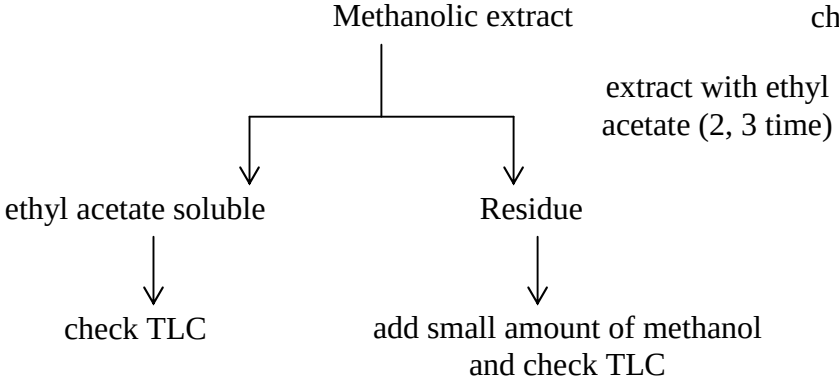
Scheme I



Scheme II



Scheme-III



Then amount of extracts were measured separately

3.2.3 Phytochemcial screening

Phytochemical screening was carried out . The different phytoconstituents in the both acetone extract and methanolic extract were identified by their colour reaction with different reagents.

Screening tests of the extract:

1. Test for volatile oils (Spot test): The methanolic extract (4ml.) was slightly concentrated and few drops of extract were spotted on filter paper. A yellow spot was persistent even after evaporation indicating the presence of oils.

2. Test for basic alkaloids: The alcoholic extract (10 ml) was concentrated to yield a residue, which was extracted with 2 % (v/v) Hydrochloric acid (3 ml). The acidic extract was equally divided into two test tubes.

a. Maeyer's test: The acidic extract when treated with maeyer's reagent (3 drops) gave a white precipitate indicating the presence of basic alkaloids.

b. Dragondorff's test: The second test solution was treated with Dragondorff's reagent (2-3 drops). No yellow or orange colour was observed indicating the absence of basic alkaloids.

c. The concentrated solution was directly spotted on TLC plate and sprayed with Dragondorff's reagent but no orange or yellow colour was observed indicating the absence of alkaloids.

3. Test for Carotenoids: The alcoholic solution was concentrated and then treated with conc. Sulphuric acid (1 ml.), orange yellow colour similar to the extract solution was developed which on long standing, turned into red indicating the presence of carotenoids.

4. Test for sterols and triterpenes (Liebermann-Burchard's test): The alcoholic solution was concentrated to yield a residue, which was dissolved in acetic anhydride (1 ml.) and chloroform (1 ml.) To this solution, conc. Sulphuric acid (2 ml.) was added from the side of the test tube without disturbing the solution. A violet ring at the junction of two liquids was observed and the upper layer was green in colour indicating the presence of triterpenes and sterols.

5. Test for Fatty Acids: The etheric solution (II) 2 ml. was concentrated and then few drops of that solution were spotted on a filter paper. A yellow spot was persistent even after evaporation indicating the presence of fatty acids.

6. Test for coumarins: The etheric solution (II) (4 ml.) was concentrated to yield a residue, which was dissolved in hot water (4 ml.). After cooling, the solution was divided into two test tubes. The first test tube was used as a control. To the second test tube, 10% (v/v) Ammonium hydrodxide solution was added drop by drop until pH8 and was then observed under ultraviolet light. Greenish yellow flurescence was observed indiating the presence of Coumarins.

7. Test for flavone aglycones: The etheric solution (II) (10 ml.) was concentrated to yield a residue, which was dissolved in methanol (4ml). The methanolic solution was equally divided into two test tubes.

a. Shinoda's test: The first test solution was treated with one small spatula of Magnesium powder in presence of conc. Hydrochloric acid (5 drops). Characteristic reddish colour was developed indicating the presence of Flavone aglycones.

b. Shibata's test: The second test solution was treated with one small spatula of zinc dust in presence of conc. Hydrochloric acid (5 drops). Characteristic reddish colour was developed indicating the presence of flavon aglycones.

8. Test for emodins (Borntiager's test): The etheric solution (2ml.) was treated with 25% (v/v) Ammonium hydroxide solution (1ml.) and was shaken vigorously. The test tube was allowed to stand for few minutes to separate two layers. The upper etheric layer was decolorized and the lower alkaline layer gained red colour indicating the presence of Emodins.

9. Test for Quinones: To the etheric solution (II) (2ml.) freshly prepared Ferrous sulphate solution (1ml.) and Ammonium thiocyanate (few crystal's) were added and treated with conc. Sulphuric acid drop by drop. Persistent deep red colour was developed indicating the presence of Quinones.

10. Test for Polyphenols (Ferric Chloride Test) : Methanolic extract (1ml.) was mixed with water (1ml.) To this solution 1% (w/v) Ferric chloride solution (3 drops) was added. A greenish blue colour was developed indicating the presence of polyphenols.

11. Test for reducing compounds (Fehling's test): The Methanolic extract (1ml) was mixed with water (1ml.). To this solution, Fehling's reagent (mixture of Fehling's reagent A and B in equal proportion) was added and then the mixture was warmed over a water bath for 30 minutes. A brick red precipitate was produced indicating the presence of reducing compounds.

12. Test for Glycosides: The methanolic extract (8ml.) was concentrated to half of the original volume and divided into two test tubes.

A. The first test solution (2ml.) was treated with 25% (v/v) Ammonium hydroxide solution (2ml.) and was shaken vigorously. Cherry red colour developed indicating the presence of glycosides (emodin glycosides).

B. Molisch's test: The second test solution was treated with molisch's reagent (5 drops) and conc. Sulphuric acid was slowly added drop by drop from the side of the test tube without disturbing the solution, violet ring at the junction of the two liquids was developed and on shaking the solution turned into violet completely indicating the presence of glycosides.

Hydrolysis of the Methanolic Extract

The remaining methanolic extract was hydrolysed by refluxing with equal volume of 10% (v/v) Hydrochloric acid for 30 minutes. After cooling, the hydrolyzed extract was re-extracted thrice with Diethyl ether (10ml.) The lower acidic layer was used for the screening test number 13. the upper combined etheric layer was dried over Anhydrous sodium sulphate and filtered. The etheric solution was used for the screening test number 14, 15, 16 and 17.

13. Test of anthocyanosides: The red acidic layer (4ml.) was basified with Sodium carbonate until basic to the litmus paper. A godrej special grey color developed gradually, but no green or blue color indicating the absence of anthocyanosides.

14. Test for anthracenosides: The etheric solution (2ml.) was treated with 25% (v/v) Ammonium hydroxide solution (1ml) and was shaken vigorously. The test tube was allowed to stand for few minutes to separate two layers. A greenish yellow color in the lower alkaline layer was observed with red colour indicating the presence of anthracenosides.

15. Test for caumarin derivative: The etheric solution (4ml.) was concentrated to yield a residue, which was dissolved in hot water (4 ml.). After cooling, the solution was divided into two test tubes. The first test tube was used as a control. To the second test tube, 10% (v/v) ammonium hydroxide solution was added drop by drop until pH8 and was then observed under UV light, yellow fluorescence in the second test tube was observed indicating the presence of caumarin derivatives.

16. Test for flavonic glycosides: The etheric solution (10 ml.) was concentrated to yield a residue, which was dissolved in Methanol (4 ml.). The methanolic solution was equally divided into two test tubes.

A. Shinoda's test: The first test solution was treated with one small spatula of magnesium powder (or small ribbon of magnesium) in presence of concentrated Hydrochloric acid (5 drops). An orange yellow color developed indicating the presence of Flavonic glysides.

B. Shibata's test: The second test solution was treated with one small spatula of Zinc dust in presence of conc. Hydrochloric acid (5 drops). No yellow color was observed indicating the absence of Flavonic glycosides.

17. Test for cardiac glycosides (Kedde's test): The etheric solution (4 ml.) was concentrated to yield a residue, which was dissolved in Methanol (2 ml.). To this solution 1 % (w/v) methanolic potassium hydroxide (1ml) and 1% (w/v) methanolic solution of 3, 5-dinitrobenzoic acid (3 drops) were added. The mixture was warmed gently. A reddish brown color was observed instead of a violet indicating the absence of Cardiac glycosides.

3.2.4 Separation of Compound by Chromatography

3.2.4.1 Thin layer chromatography

It is the special application of the adsorption chromatography which contains the solid (silica gel) as stationary phase and liquid as mobile phase. The solvent moves up the plate by capillary action. Visualization of spots can be done either by observing the plate under UV light or by use of spraying reagents.

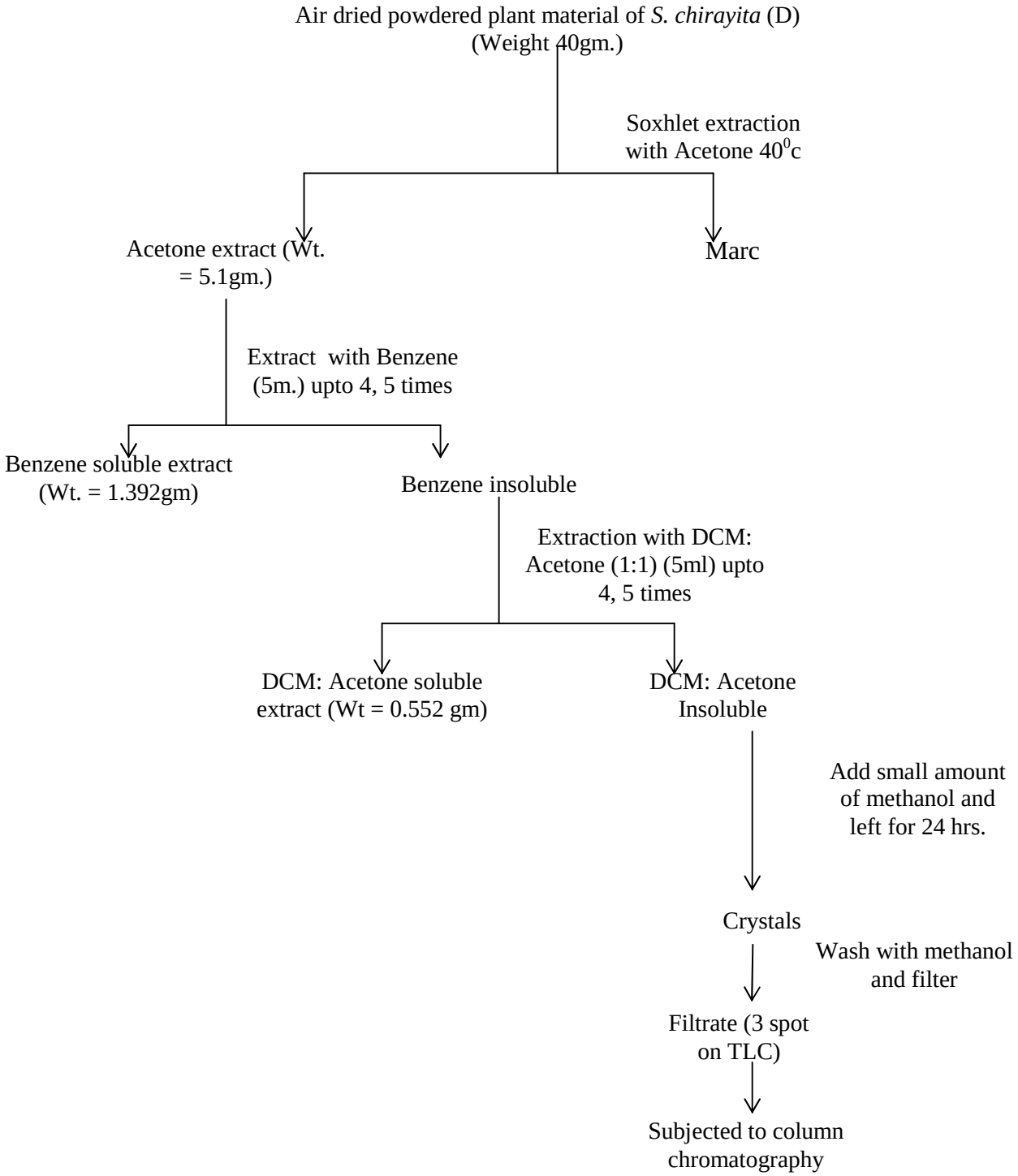
Rf-values and colours of some prominent spots along with the marker in the test solution track are given to assist identification of drug.

Here different solvent systems were used for different fraction. For Acetone and Benzene fraction, 0.1% MeOH in chloroform was used and for ethyl acetate fraction ethyl

acetate: methanol: water (21.3: 2.2: 10.5) was used. And their Rf values were calculated separately.

$$Rf = \frac{Distance\ travelled\ by\ spot}{Distance\ travelled\ by\ the\ solvent\ front}$$

3.2.4.2 Column Chromatography



The remaining extract was made dry and chromatographed over silica gel (60-200 Mesh LR) packed column eluted with chloroform. The column was eluted subsequently with 1%, 2%, 3%, 5%, 10%, 20%, 25% and 30% methanol in chloroform. Each eluent was monitored under TLC plate. The sequences of eluents are tabulated as follows:

Table 1. Sequences of Eluents

S.N.	Eluents	Effluent fractions No.	Weight of isolated substance	Remark
1	Chloroform	1-4	-	No spot on TLC
2	1%Methanol in chloroform	5-9	-	,,
3	2% Methanol in chloroform	10-15	-	,,
4	3% Methanol in chloroform	16-20	-	,,
5	5% Methanol in chloroform	21-25	8mg	Single spot on TLC, yellow color (DI)
6	10% Methanol in chloroform	26-30	-	No spot on TLC
7	20% Methanol in chloroform	31-35	-	,,
8	25% Methanol in chloroform	36-42	9mg	Single spot on TLC yellow colour (DII)
9	30% Methanol in chloroform	43-50	-	No spot on TLC

CHAPTER FOUR: RESULTS

4.1. Ethnomedicinal Study of *Swertia chirayita*

Ethnomedicinal study was carried out in the study area under following headings:

4.1.1 Ethnomedicinal uses of plant part(s) of *Swertia chirayita*

During the field visit, local people answered different view for the question "which part of Chiraito (*Swertia chirayita*) do you use for medicinal purpose?". Out of total forty number of local people, who were interviewed during field visit, 65% people used whole plant for medicinal purpose, 20% people were involved in using root and 15% of people involved in using leaf and inflorescence.

4.1.2 Commonly Used forms of Medication of *Swertia chirayita*

Most of the people used the plant as decoction. Some of them used the plant as juice and some used the plant directly.

Decoction: It is generally obtained by boiling plant/part(s) in the water and filtered through muslin cloth.

Juice: The plant/part(s) are crushed with or without adding water sqeezed and filtered through muslin cloth.

Directly: Some people used directly for themselves and for cattle.

4.1.3 Uses of *Swertia chirayita* in Certain Disease(s)

In the study area Chiraito was used to cure different diseases like Kukhat (Typhoid), Jworo (fever), Gano gayako (gastritis), Sakti bardak (Tonic), Juka (Worms), Madhumeh (Diabetes). For different diseases different forms of medication was used. For Typhoid, fever and gastritis decoction method was carried out, for diabetes and worms juice was used and plant was directly used for buffalo, goat etc. as tonic.

4.1.4 Exploitation of *Swertia chirayita*

The exploitation of medicinal plants from different sites of Nepal is very common. During the field visit, it was not found dense population of the Chiraito. It was found that these plants were slowly being taken away by the people. So that the plants are disappearing. According to villagers, the main cause of disappearing of plants was by open grazing and immature harvesting.

4.1.5 Involvement of Different Class in Collection of Plant Materials

During the field visit it was found that most of the local people from deprived class are dependant upon forest resources as they have low land and low economic status. Almost 85% people from deprived class were involved in collection of plant resources (including Chiraito) while there were only 15% involvement of local people from privileged class. Among them majority of women (upto 58%) from deprived class and (11%)from privileged class were involved.

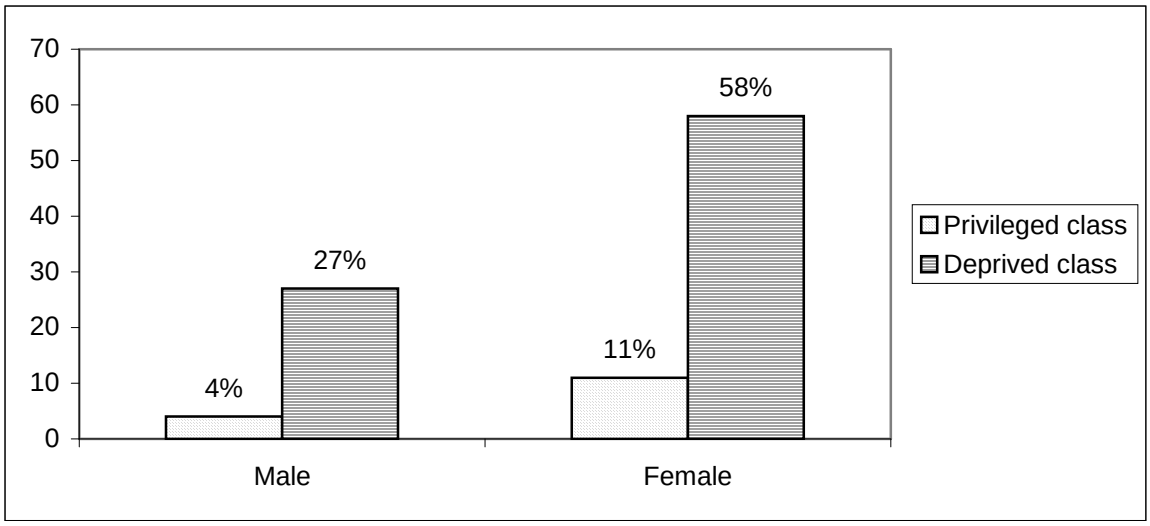


Fig. 2: Participation of Gender and Class in Collection of Plant Resources

4.1.6 Conflict on Benefit Sharing Among Stakeholders

The collectors were not able to obtain proper benefit from trading of *Swertia chirayita* due to big margin by middleman and traders. In the present study it was found that the ratio of price among collectors, middleman and trader is 1:2:4(Rs 80:160:300). So this result shows injustice on benefit sharing. There was highly involvement of women and deprived groups in the collection of plant material but they had least benefit on trading it. So deprived groups and women raise the voice of discriminations. This scenario causes conflict between them.

4.1.7 Availability and Conservation Status of *Swertia chirayita*

According to respondent there was found less amount of *Swertia chirayita* than before. The reasons for this may be due to immature harvesting, open grazing and over exploitation.

Due to lack of proper knowledge about harvesting, they collect plant before maturity. As a consequence *S. chirayita* has been getting threatened. Local healers are found to be the key figures that provide health care service in the study area. They have broad indigenous knowledge about various uses of plants but the knowledge kept secret. They believe that if they communicate knowledge related traditional medicinal practices to others the healing power of medicine and their mythological belief would be less-effective. It was found that dependency of local people on medicinal plant is higher in people from deprived community. There are two possibility beside this outcome; one is they are rich in indigenous knowledge about medicinal plant and another is they have low economic status and therefore cannot afford allopathic medicine.

4.2 Phytochemical screening

4.2.1 Estimation of Soluble Extracts Using Different Organic Solvent

The estimation of extracts with different organic solvents have been carried out by using soxhlet apparatus.

The acetone extracts of all samples were taturated separately with benzene. The highest benzene soluble fraction was obtained from the acetone extract of *S. chirayita* (D) followed by *S. ciliata* and *S. nervosa*.

The benzene soluble fraction of all samples were further extracted with Dichloromethane: acetone (1:1).

The highest dichloromethans: Acetone soluble fraction was obtained from the benzene extract of *S. chirayita* (C) followed by *S. chirayita* (D) and *S. angustifolia*.

Table I: Estimated Values of Different Extracts with Different Organic Solvents

S.N.	Name of species	Percentage of extracts	
		Acetone	Methanol
1	*S. ciliata*	11	3.05
2	*S. nervosa*	8.71	12.42
3	*S. angustifolia*	11	15.01
4	*S. chirayita (A)*	6.8	9.63
5	*S. chirayita (B)*	6.33	8.33
6	*S. chirayita (C)*	10	7.06
7	*S. chirayita (D)*	12.75	10

The methanolic extract of all samples were also further extracted with ethyl acetate. The highest ethyl acetate soluble fraction was obtained from the methonolic extract of *S. chirayita* (D) followed by *S. chirayita* (A) and *S. ciliata*.

Table II: Estimated Values of Different Extracts with Different Organic Solvents

S.N.	Name of species	Percentage of extracts		
		Benzene	DCM: acetone	Ethyl acetate
1	*S. ciliata*	3.43	0.85	2.26
2	*S. nervosa*	3.3	0.82	1.35
3	*S. angustifolia*	2.5	1.35	1.42
4	*S. chirayita (A)*	1.7	0.62	3.2
5	*S. chirayita (B)*	1.75	0.57	2.16
6	*S. chirayita (C)*	2.88	1.45	1.01
7	*S. chirayita (D)*	3.48	1.38	4.25

Joshi (2003) reported about 4% of the active bitter constituents in inflorescence of S. chirayita (from Maipokhari Ilam) however, in the present investigation it was found to contain about 4.5% which was slightly higher than reported value.

4.2.2 Phytochemical Screening

The phytochemical screening of different species of *Swertia* were performed. The results obtained for screening test of methanolic and acetone extract are presented in table below:

Table III: Phytochemical screening results are tabulated as follow

S.N.	Tests	*S. ciliata*		*S. nervosa*		*S. angustifolia*		*S. chirayita (A)*		*S. chirayita (B)*		*S. chirayita (C)*		*S. chirayita (D)*	
		Aceton	MeOH	Aceton	MeOH	Aceton	MeOH	Aceton	MeOH	Aceton	MeOH	Aceton	MeOH	Aceton	MeOH
1	Volatile oils	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Basic alkaloids	-	+	+	-	-	-	+	+	+	+	+	+	+	+
3	Carotenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Sterol & triterpenes	+	-	+	+	+	+	+	+	+	+	+	+	+	+
5	Fatty acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Caumarins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Flavon aglycones	-	+	-	+	+	-	+	+	+	+	+	+	+	+
8	Emodins	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Quinones	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	Polyphenols	-	+	+	+	-	-	+	+	+	+	+	+	+	+
11	Reducing compd.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	Anthocyanosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	Anthracenosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Caumarin derivative	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	Flavonic glycosides	-	+	+	+	+	+	+	+	+	+	+	+	+	+
17	Cardiac glycosides	-	+	+	-	-	+	+	+	+	+	+	+	+	+

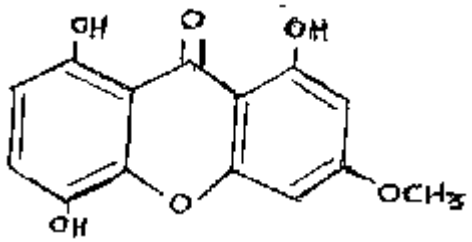
On the basis of phytochemical screening it was found that the plants are rich in natural compounds like; glycosides, flavon aglycones, carotenoids, sterol and triterpenes, carnarins, polyphenols, basic alkaloids, fatty acids, quinone, and reducing compound.

.2.3 Isolation and Characterization

4.2.3.1. Compound No. DI

Compound DI was isolated as yellowish amorphous solid from acetone extract of *S. chirayita* (D) by silica gel column chromatography. It was further recrystalised by methanol. The fractions NO. 21-25 from column chromatography eluted by 5% methanol in chloroform were combined on the basis of their Rf-values on TLC and concentrated.

On the basis of 'H-NMR-, ^{13}C-NMR, COSY, HMBC and HMQC data the compound was assigned as 1, 5, 8-trihydroxy-3-methory xanthone (bellidifolin). Finally the spectral data were compared with that of the reported data, K. Ishimura *et al.* (1990) and it matched.



1, 5, 8-trihydroxy-3-methoxy xanthone (bellidifolin)

The Rf-value was found 0.15 (sovent system 0.1% methanol in chloroform) and it is soluble in methnol, chloroform and aceton

4.2.3.2 Compound No. D_{II}

Compound DII was isolated as yellowish amorphous solid from acetone extract of *S. chirayita* (D) by silica gel column chromatography. The fractions No. 36-40 from column chromatography obtained on eluting by 25% MeOH in $CHCl_3$ were mixed on the basis of their Rf- value son TLC and concentrated to get compound DII.

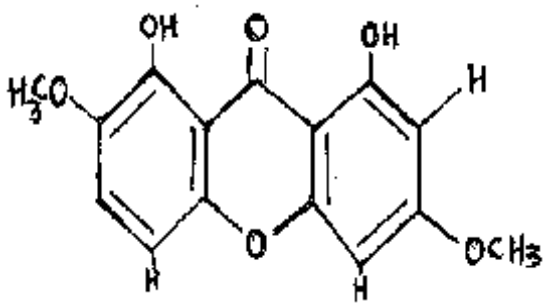
The compound No. DII was found similar to compound MM_{73} (methyl swertianine) isolated by Manandhar *et al*. (1996).

Table IV: Identification of Compound D_{II} by CO-TLC

Compound	Rf value
MM73	0.73
DII	0.73
M73+DII	0.73

Solvent system; 0.1% MeOH in chloroform.

Thus Co-TLC of the isolated compound with that of authentic sample MM_{73} was checked and found same. On the basis of spectral data (UV-spectrum, IR-spectrum and H-NMR-Spectrum) and CO-TLC, the isolated compound DII was identified as 1, 8-dihydroxy-3, 7 dimethoxy xanthone (Methyl swertianine).



1, 8-Dihydroxy-3, 7-dimethoxy xanthone (methyl swertianine)

Rf-value of methyl swertianine was 0.73 (0.1% MeOH in chloroform) and soluble in warm hexane, EtOAc, MeOH and chloroform.

4.2.4 Observation of Isolated Compound DI and DII in other Samples

Presence of compound DI and DII in acetone extract and benzene extract were determined by comparing Rf-value in Thin layer chromatogram.

Rf-values of different sample in different organic solvent are given below:

Table IV: Presence of Compound DI in Other Samples

S.N.	Name of samples	Rf-value of	
		Acetone extract	Benzene extra
1	Compound D_I	0.130	0.150
2	*S. ciliata*	0.132	0.151
3	*S. nervosa*	0.133	0.147
4	*S. angustifolia*	0.128	0.145
5	*S. chirayita (A)*	0.130	0.150
6	*S. chirayita (B)*	0.130	0.150
7	*S. chirayita (C)*	0.130	0.150
8	*S. chirayita (D)*	0.130	0.150

Solvent system – 0.1% MeOH in chloroform.

Table V: Presence of Compound D_{II} in Other Sample

S.N.	Name of samples	Rf-value of	
		Acetone extract	Benzene extra
1	Compound D_{II}	0.730	0.730
2	*S. ciliata*	0.735	0.733
3	*S. nervosa*	0.730	0.730
4	*S. angustifolia*	0.725	0.727
5	*S. chirayita (A)*	0.730	0.730
6	*S. chirayita (B)*	0.730	0.730
7	*S. chirayita (C)*	0.730	0.730
8	*S. chirayita (D)*	0.730	0.730

Solvent system – 0.1% MeOH in chloroform.

4.2.5 Observation of Amorogentin and Amoroswerin

To extract bitter principle of plant, the methanolic extract was further extracted with ethyl acetate, Joshi (2003).

The reported Rf-value of amarogentin = 0.5 and amaroswein = 0.44 with solvent system ethyl acetate: methanol: water (21.3: 2.2:10.5), Indian Herbal pharmacopoeia Revised New edition 2002.

Rf-value of each ethyl acetate extracts were recorded with solvent system ethyl acetate: MeOH: water (21.3: 2.2:10.5).

Table VI: Observation of Amarogentin and Amaroswerin in the Other Sample

S.N.	Ethyl acetate extract of species	Rf values	
		Amarogentin	Amaroswerin
1	*S. ciliata*	-	-
2	*S. nervosa*	0.512	-
3	*S. angustifolia*	0.512	-
4	*S. chirayita (A)*	0.512	0.439
5	*S. chirayita (B)*	0.500	0.439
6	*S. chirayita (C)*	0.512	-
7	*S. chirayito (D)*	0.500	0.439

The observed Rf-value were matched with that of reported Rf-value under the condition of same solvent system. Amarogentin is only absent in *S. ciliata* and amaroswerin is present in *S. chirayita* (A), *S. chirayita* (B) and *S. chirayita* (D).

CHAPTER FIVE: DISCUSSION AND CONCLUSION

5.1 Ethnomedicinal Study

In the present study ethnomedicinal uses of medicinal plant, Chiraito, have been studied. All the information presented here were based on field observation, interview with local people and knowledgeable people. In the study area different ethnic groups include Brahmin, Kami, Gharti, Sarki, Magar, Damai, Sunar and others.

Comparative study between the uses of present findings and previous findings has been done. Previous findings have been given in appendix-IV.

In the previous finding, use of *S. chirayita* was found in following diseases; Malaria, pneumonia, fever, abdominal disorder/pain, headache, throat pain, chest pain, cough, diarrhoea, worms, jaundice, typhoid in different sites of Nepal but present study shows the uses of chiraito in typhoid, fever, gastritis, as tonic, worms and diabetes.

In the present study, it was found that whole plant, root and leaf along with inflorescence were taken seperately for medicinal purpose. But Gurung (2003) reported the uses of Chiraito in different diseases by taking whole plant, and Pathak (2005) reported the uses of Chiraito in fever and worms by taking leaf and stem.

In the present study, *S. chirayita* was used by most of the people to treat fever, worms, gastritis and diabetes. KC and Chaudhary (2006) described its use in Typhoid and diarrhoea. Bhattarai (2002) described its uses in fever and jaundice by Bhotiya and Sherpa communities around Makalu Barun National Park and Buffer zone.

KC and Chaudhary (2006) reported the form of medication of Chiraito was decoction while our study showed similar job about the medication of Chiraito except its direct use by local people for themselves and for buffalo and goat.It was found that *S. chirayita* was used as tonic for cattle in the study area.

In the study area unequal benefit sharing has been found and the collectors always fail to generate a significant proportion of advantages due to absence of institutional, technological, organizational and informational constraints.

Present study shows unequal benefit sharing among collectors, middle man and trader with proportion 1:2:4. K.C. and Chaudhary (2006) reported the proportion 1:2:3. It shows injustice on benefit sharing.

According to Subedi and Ojha (2001), one village trader's profit is 58 times higher than that of a harvester.

During field visit it was observed that more uneducated people, mostly from deprived class and women were involved in the collection of the plant material. Not only do they harvest randomly but they use plant for cattle by open grazing system which cause tremendous decrease of population of plant in that area.

The indigenous knowledge has been found more in people above 40 and less among the young generation. This indicate that the indigenous knowledge has not been handed over for generation to generation properly. Most people were interested to cultivate this plant but they don't know about its cultivation process,actual market, chemical composition in plant etc.

Modern medicinal science has emphasized allopathic medicine and several different brands have been developed for each disease. Many of these medicines have to be imported from other countries. In the study area, Pokharathok, there were different ethnic group who were enriched in indigenous knowledge about plant. So they do not have to depend on allopathic medicine. According to local people in study area, these herbal medicines are very useful for curing specific diseases and do not have side effect as in allopathic medicines.

5.2 Phytochemical screening

5.2.1 Variation of Extracts in Different Solvent System

S. chirayita (D) showed higher yield value with solvent acetone while *S. angustifolia* showed highest yield with solvent methanol. Benzene and ethyl acetate extract of *S. chirayita* (D) showed good percentage of yield while DCM: acetone extract of *S. chirayita* (D) showed higher percentage of yield. These variation may be created due to the impact of environment since the samples were collected from different sites of Nepal.

Joshi and Joshi (1998) reported that same plant shows chemically and morphologically different responce under influence of environment and altitude.

Pant *et al*. (2000) reported the different extracts of *Swertia* have different biological activity viz. ethyl acetate extracts have antifeedant property, Benzene extract have anti-inflamatory activity, acetone fraction have hypoglycemic activity, methanolic and dichloromethane extract has antifungal activity.

These chemical variation may be due to the impact of environment. Since the samples were collected from different sites.

5.2.2 Phytochemical Screening of Different Swertia

Phytochemical screening of different species of *Swertia* showed the presence of fatty acid, basic alkaloids, carotenoid, sterol and triterpene, caumarin, flavon aglycon, quinone, polyphenols, Glycosides. *S. ciliata, S. nervosa, S. angustifolia, S. chirayita* are the medicinal plants of the same family, Gentianaceae. However, polyphenol and basic alkaloid were present in other species of *Swertia* except in *S. angustifolia*.

5.2.3 Presence of Compound D_I and D_{II} in Other Samples

Thin layer chromatogram of all sample showed the presence of compound D_I and D_{II}. This issue was observed by checking the compound on TLC with solvent system 0.1% MeOH in chloroform (Table IV and V).

There was found slightly fluctuation of Rf-value in the *S. ciliata, S. nervosa* and *S. angustifolia*. This fluctuation may be due to the impact of environment. Since samples were taken from variety of sites. Harborne (1962) showed that minimum amount of an enzyme changed for the alteration of flavonoid structure Amarogentin is absent in *S. ciliata* and Amaroswerin is absent in *S. ciliata, S. nervosa, S. angustifolia & S. chiraryita* (c). Kenney *et al.* (1973) reported that amaroswerin and amarogentin are the bitter principles of *S. chirayita*.

5.2.4 Compound D_I and D_{II}

From the analysis of spectral data the compound DI was assigned as 1, 5, 8-trihydroxy-3-methoxy xanthone (bellidifolin) and compound DII was assigned as 1, 8-Dihydroxy-3, 7-dimethoxy xanthone (methyl swertianine).

One of the chief constituents of *Swertia spp.* are xanthones. They are found as aglycon or as glycosidic forms, combining with common sugar unit like glucose and

rhamnose. Present study showed new form of xanthone. Some xanthone and xanthone glycoside isolated from various *Swertia spp*. are enumerated in Appendix-V

CHAPTER SIX: RECOMMENDATIONS

In order to preserve indigenous knowledge, as well as to increase population of Chiraito following recommendation has been suggested on the basis of this study.

Indigenous knowledge of local people should be investigated:

There was seen generation gap about the traditional practice on medicinal plant, Chiraito. Young generations do not seen interested and the knowledge enriched people do not want to open the secret of their tradition to others. If it is not investigated, the typical traditional practice won't be preserved.

Selected species of Chiraito should be provided for cultivation:

Local people should be encouraged to cultivate selected species of Chiraito, *S. chirayita* from Solma,Terhathum, which have high yield of its active chemical constituents. And to improve economic status of deprived class, government should provide land for cultivation.

Immature harvesting and open grazing system should be strictly prohibited:

During the field visit, it was found that most people were involved in immature harvesting and open grazing which cause the tremendous decrease in plant population. Such activities should be stopped.

Create awareness regarding conservation, cultivation and utilization:

The intellectual and knowledgeable people should initiate the cultivation practice on Chiraito. They have to make a trend about cultivation because cultivation is the best mode of conservation. This cultivation practice will not only help them to utilize the land but at the same time plant should be grown in large scale which will help them to raise economy. Unequal benefit sharing create conflict among stakeholders. So this equity issues should be studied.

The main bitter constituents, Amorogentin and amaroswerin, were found in *Swertia chirayita* but it was absent in *S. ciliata*, *S. angustifolia* and *S. nervosa*. So *Swertia chirayita* could be recommended for further detailed study upto molecular level for its bitter constituents.

Detailed chemical study of other species of *Swertia* should be carried out.

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Fig 01

Fig 02

Fig 03

Fig 04

Fig 05

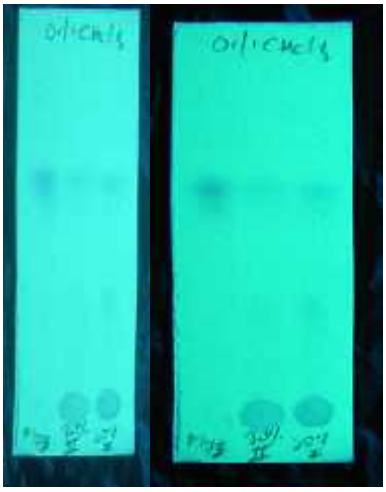


Fig 06

Fig 07

Fig 08

Fig 09

Appendix-II

Questionnaire

Questionnaire Used for Household Survey

1. VDC, ward No:
2. Village/Tole:
3. Name:
4. Sex:
5. Age:
6. Education status: Literate ☐ Illiterate ☐ Educated ☐
7. Occupation:
8. Family Size:
9. What is the name of plant in local term?
10. Which part do you use for medicinal purpose?
11. For which disease do you use the plant?
12. What is the local methodology of use?
13. When and how do you collect the plant?
14. Do you cultivate this plant or do you want to cultivate this plant?
15. Is there proportion of benefit sharing equitable among collectors of all categories, class, caste, ethnicity, gender? If not please explain why?
16. How much rupees do you get per kilogram of chiraito?
17. Do you have any suggestions regarding the better management and sustainable utilization of plant?

Appendix-III

Table 1: Involvement of Local People for Using Different Plant Parts

Respondents						Total
WP*user	%	R* user	%	L-*8.inf.user	%	Respondents
26	65	8	20	6	15	40

Source: Field survey/ sample survey 2005-2006.

*WP = Whole plant, R = root, Linf = Leaf inflorescence.

Table 2: Participation of Users Group in Collection of Plant

Privileged class		Deprived class		Total (%)
Male (%)	Female (%)	Male (%)	Female (%)	
2 (5%)	4 (10%)	10 (25%)	24 (60%)	40 (100%)

Source: Field survey/ sample survey, 2005-06.

Table 3: Population of Study Area

	Arghakhanchi	Pokharathok, VDC
Total population	208391	3928
Total household	40869	736
Total male	96349	1843
Total female	112042	2085

Source: Statistical year book, 2005.

Table 4: Ethnic Composition of the Study Area

Castes	Gharti/Bhujel	Yadav	Magar	Sarki	Kumal	Brahmin-hill	Damai/Dholi	Others	Kami	Sunar	Chhetri	Sanyasi
No.	131	5	105	94	5	984	79	11	795	70	241	8

Source: Population census of Nepal, 2001.

Appendix-IV

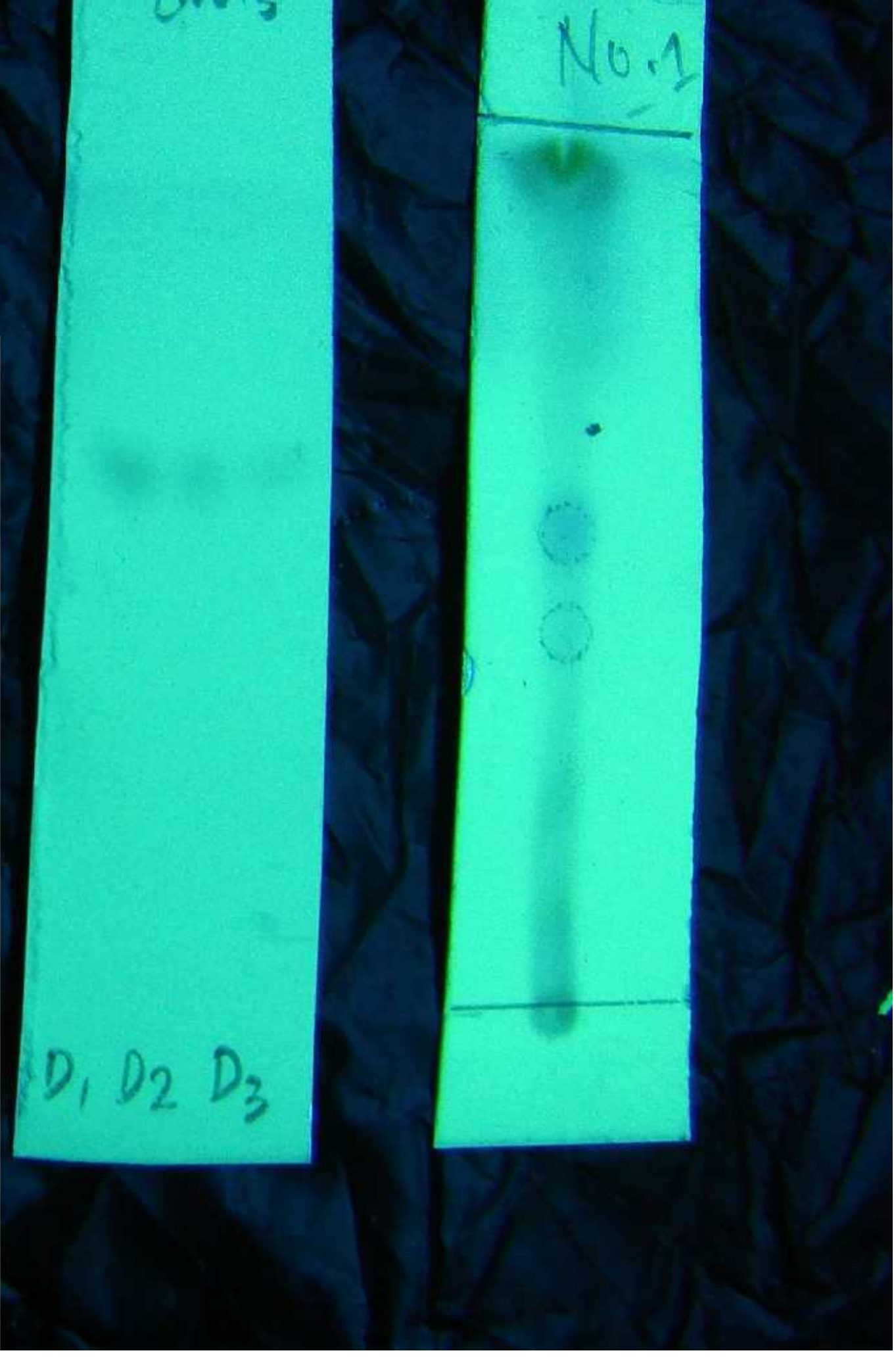
S.N.	Study area	Researcher	Year	Indication (use of chiraito)	Plant part
1	Gwalek VDC, Baitadi	Devkota, Ranju and S.B. Karmacharya	2003	Malaria, Pneumonia, Fever and abdominal pain	WP
2	Tinjure, Terhathum	Gurung, Khilendra	2003	Fever, headache, throat pain, chest pain	WP
3	Tapethok VDC, Taplejung	Oli, Bheshraj	2003	Fever, Pneumonia, cough, diarrhoea	WP
4	Dolpa, Jumla	Kunwar, Ripu and Narayan PS Duwadea	2003	Fever	WP
5	Bhagawati VDC, Darchula	Panta SR and IR Panta	2004	Malaria, fever, cough abdomen disorder	WP
6	Thumpokhara VDC, Sindhupalchok	Rai et al.	2004	Fever, headache and malaria	WP
7	Daman	Pathak, Loknath and SR Pandey	2005	Fever, worms	LS
8	Makalu Barun National Park and Buffer Zone	Bhattarai, M.	2002	fever, jaundice	WP
9	Resunga, Gulmi	KC and Chaudhary	2006	Diarrhoea	WP

WP = Whole Plant, LS = Leaf, Steam

Appendix V

Name of species	Name of xanthone	Reference
(i) *S. japonica*	Swertianol, and Swertianolin , Billidofolin, nor-swertianolin and iso swertianolin, 8-0-B glucosyl-1, 3 dihydroxy-5 methoxy xanthone	Sashina *et al*. (1942) Sakamoto *et al*. (1982) Gnosal *et al* (1980)
(ii) *S. tosaensis*	Swertianol and swertianolin	Nakaoki T., Hido Y. (1943)
(iii) *S. chirayita*	Swertianolin, Billidifolin Swertinin, Swerchirin, Gentiakochinanine, Mangitonin, decussatin, swertianin, 1, 3, 5, 8-tetrohydroxy xanthone, 1, 3, 7, 8-tetrohydroxy xanthone	Dolal S.R. and Shah R.C. (1956)
(iv) *S. panniculata*	Swertianol, Billidifolin, and Swerchirin. Decussatin	Prakash *et al*. (1982) Sakai *et al*. (1981)
(v) *S. mussotti*	Swrtianol, Swerchirin and Dedussation. Mangiferin	Dingjing, Y, Sun Hung Fa (1980)
(vi) *S. angustifolia*	Billidifolin Swerchirin, Decussatin, 1, 3, 4, 5, 8-Pentamethoxy xanthone, 1, 3, 4, 7, 8-pentamethoxy xanthone and, 1-0-B-D-glucopyranosyl-3, 7, 8trihydroxy xanthone	Ahmad *et al*. (1977) Gnosal *et al*. (1978)
(vii) *S. alata*	Bellidifolin	Ali *et al*. (1979)
(viii) *S. decussata*	Swertinin	Bhan *et al*. (1953)
(ix) *S. mileensis*	Swerchirin and Decussatin	He R., Feng S., Nie R. (1982)
(x) *S. iberica*	Decussatin, Swertianin, Swertiaperenin, Swertiaiberin, Gentiakochinanin,	Solovera E.U., Glgzin V.I. (1980)

	Isogentiakochinanin, 1-0-B-D-glucopyranosyl-3, 7, 8-trihydroxy xanthone and 1-0-Prime verosyl-3, 7, 8 trimethoxy xanthone	Solovera EU, Glyzin V.I. (1980)
(xi) *S. ciliata*	Swertianolin	Animad *et al*. (1973)
(xii) *S. purpurascens*	Nor swertionolin, Isowertiaanolin	Ahmad *et al*. (1973)
(xiii) *S. corodata*	Mangiferin	Khan M.I., Hoggani M.H. (1981)
(xiv) *S. perfoliata*	Mangiforin	Haggani M.H. (1981)
(xv) *S. hookeri*	1-0-stearyl-3, 5-dimethoxy xanthone	Gnosal *et al*. (1980)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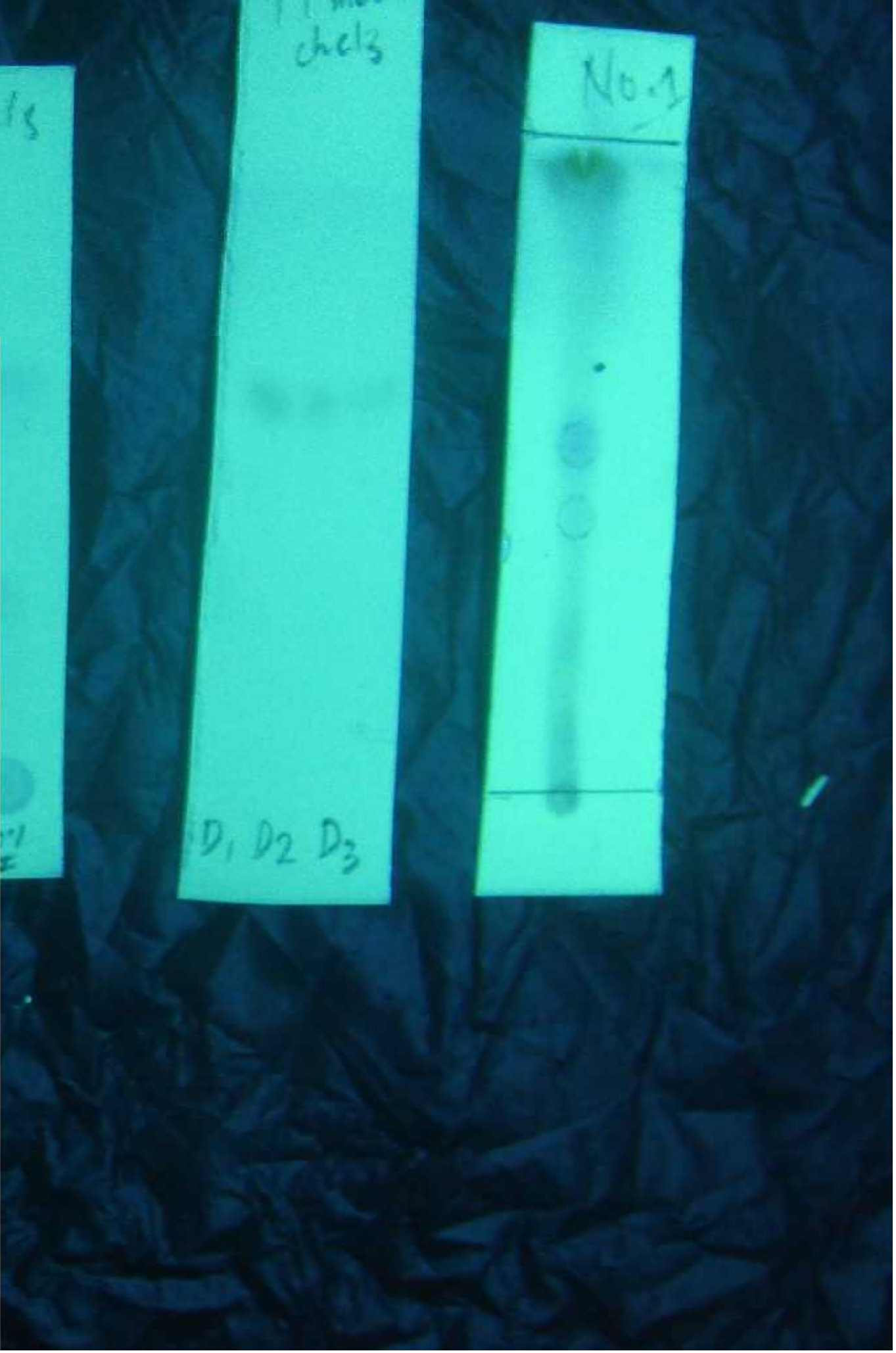
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D1 D2 D3


73
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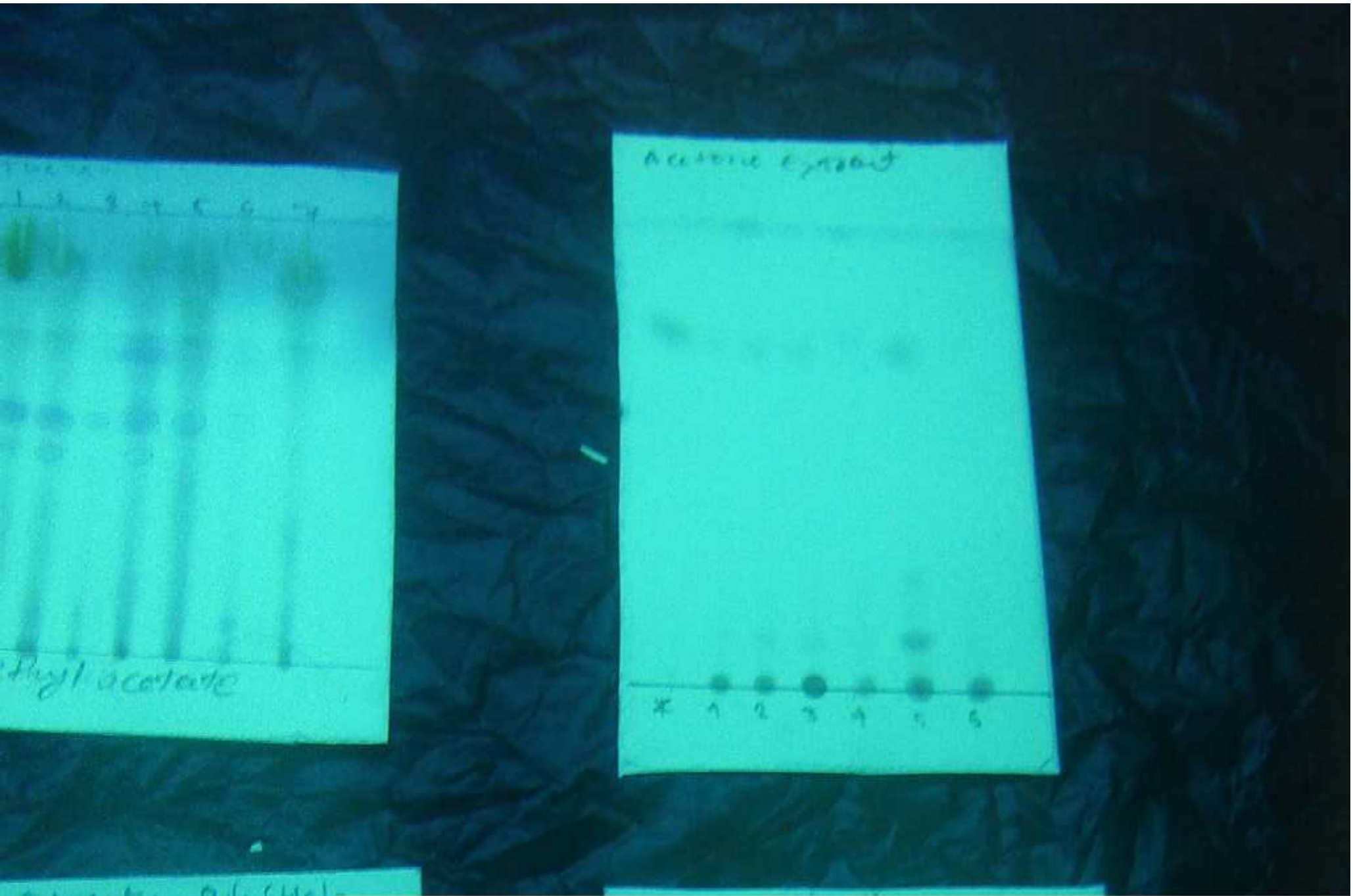
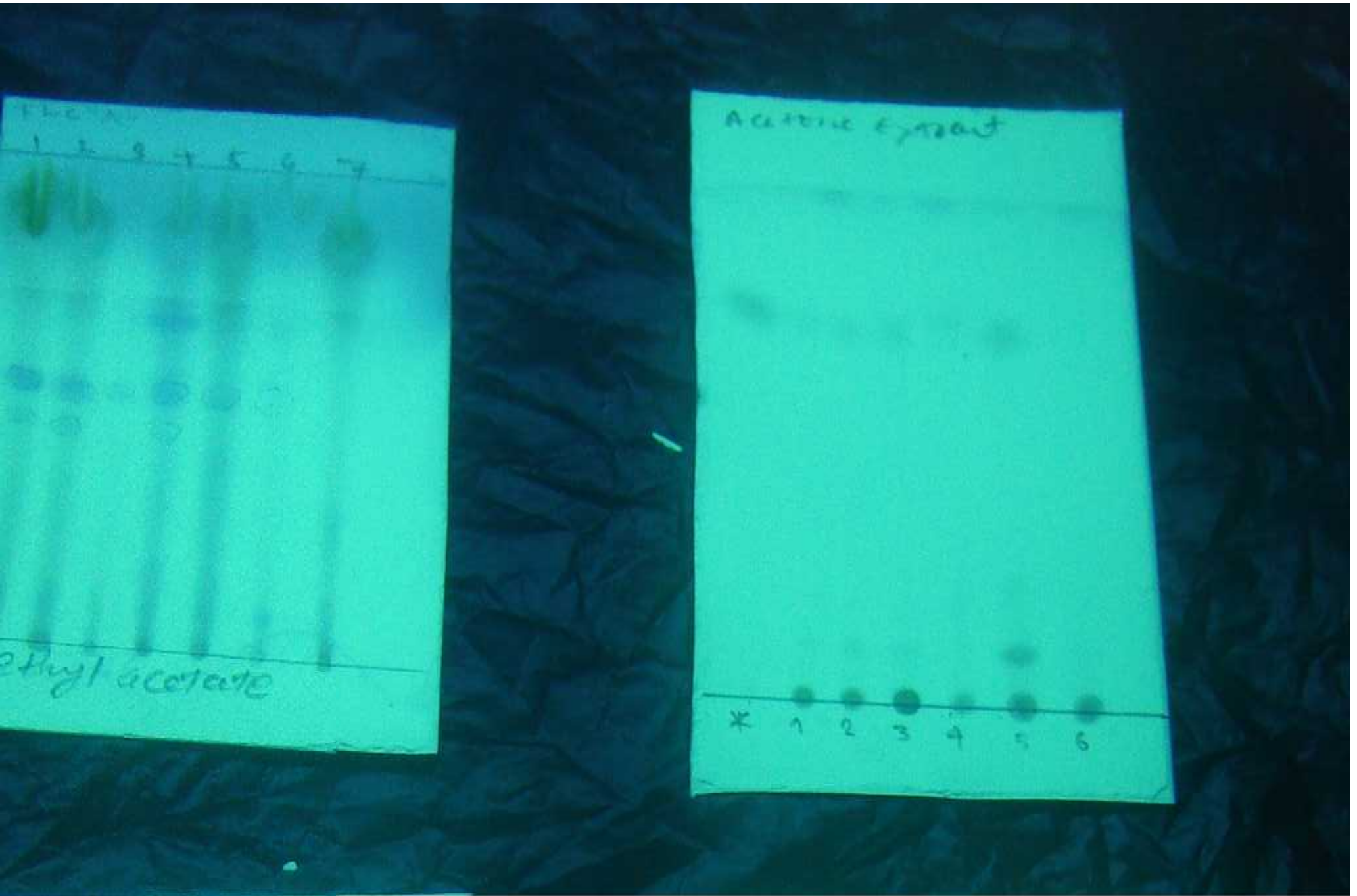

Acetone extract
* 1 2 3 4 5 6


1 2 3 4 5 6 7
ethyl acetate
Acetone extract
* 1 2 3 4 5 6

चिराईतो

वैज्ञानिक नाम:	***Swertia chirayita* (Roxb. ex Fleming) Karstrn**
नेपाली नाम:	चिराइतो, तीते
संस्कृत नाम:	चिरतिक्त, किरातक
हिन्दी नाम:	चिरायता
अंग्रेजी नाम:	Chireta
अन्य नाम:	चरैतो, चिराइतो, चिरैता, तिते, र तिक्ता ।