Journal of Plant Resources (2024) **22**(1), 72-79
https://doi.org/10.3126/bdpr.v22i1.68345

Pharmacological Activities of Some Medicinal Plants of Nepal

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Abstract

Nepal has the most unique and valuable medicinal plants, owing to its diverse climatic conditions. Plant extracts as traditional medicine provide primary health coverage for about 65-80% of the world's population, especially in the developing world. However, very limited biological research has been done in Nepal to date. This research aims to evaluate the pharmacological activity of eight medicinal plants from Nepal namely *Anaphalis triplinervis* (Sims) C.B.Clarke, *Chlorophytum arundinaceuum* Baker, *Curculigo orchioides* Gaertn., *Girardinia diversifolia* (Link) Friis, *Rhododendron anthopogon* D. Don, *Swertia racemosa* (Griseb.) Wall. Ex C.B. Clarke and two lichen species *Dolichousnea longissima* (Ach.) Articus and *Hypotrachyna cirrhata* (Fr.) Divakar, A. Crespo, Sipman, Elix & Lumbschsp. The ethanolic extracts of these plants were prepared by cold extraction process after which acute oral toxicity, antidiarrhoeal and antiulcer activity were analysed. All laboratory works were done in Natural Products Research Laboratory, Thapathali, Kathmandu, Nepal. The acute oral toxicity study showed no morbidity, mortality, toxicity signs, or symptoms at 2000 mg kg^{-1} in all medicinal plants except *H. cirrhata*. In the anti-ulcer test, *C. orchioides, D. longissima, G. diversifolia, H. cirrhata* and *R. anthopogon* significantly reduced gastric lesions in the ethanol-induced ulcer model at 500 mg kg^{-1} when compared to the standard drug, sucralfate (100 mg kg^{-1}). The antidiarrheal effect was evaluated by gastrointestinal motility test at 500 mg kg^{-1} body weight in mice where the extracts of *H. cirrhata and S. racemosa* showed considerable antidiarrheal effect by reducing GI motility significantly compared to their respective control and standard drug loperamide (2 mg kg^{-1}).

Keywords: Acute toxicity, Antidiarrheal, Anti-ulcer, Extract preparation, Medicinal value

Introduction

Plants have been used for special medicinal purposes since ancient times. Plant extracts as traditional medicine provide primary health coverage for about 65-80% of the world's population, especially in the developing world (World Health Organization [WHO], 2002). By the end of the twentieth century, 170 herbal drugs got official recognition for disease control (Alamgeer et al., 2018). According to the WHO, 75% of the world's population uses phytotherapeutic agents for the treatment and prevention of various diseases and 11% of drugs among the essential drugs are of plant origin (Pan et al., 2014; Rates, 2001)

According to the Organization for Economic Co-operation and Development (OECD), judgment of oral acute toxicity testing of any sample is based on biometric evaluations depending on the mortality or the moribund status of the animals observed for 14 days (periodically first 24 hours with special attention given during the first 4 hours) after oral administration of test sample at a defined dose in laboratory animal (Falya et al., 2020; OECD, 2022). It allows for the determination of LD50 the substance using mortality in animals as the main observational endpoint and helps in ranking of the substance for classification purposes and hazard assessment. This test is usually carried out before testing for further pharmacological activities and helps to develop new drugs by determining the therapeutic potential of a drug molecule (Falya et al., 2020; Khan & Akhtar, 2012).

Peptic ulcer is among the most predominant gastrointestinal diseases in the world and is characterized by ulceration in the stomach and duodenum. The pathophysiology of ulcers is due to an imbalance between gastric aggressive factors (e.g., acid, pepsin, infection and non-steroidal anti-inflammatory agents) and mucosal defensive factors such as mucus bicarbonate and blood flow (Brooks, 1985). The treatment of peptic ulcers is still unsatisfactory as many of the drugs used may produce

many adverse reactions like arrhythmias, impotence, gynaecomastia and hematopoietic changes (Akhtar & Ahmad, 1995). Medicinal plants, being easily available and cheaper, can show promising anti-ulcer activity (Bhattacharya et al., 2007)

Diarrhea is one of the most common causes of millions of deaths every year (Field, 2003). It may be infectious (viral, bacterial, protozoal) or non-infectious (Thiagarajah et al., 2018). In modern research, identification of new sources of antidiarrheal effects has become one of the primary focus.

The present study was undertaken to evaluate the toxicity, anti-ulcer, and anti-diarrhoeal activity of the ethanolic extracts of eight different plants namely *Anaphalis triplinervis* (Sims) C.B.Clark, *Chlorophytum arundinaceuum* Baker, *Curculigo orchioides* Gaertn., *Dolichousnea longissima* (Ach.) Articus, *Girardinia diversifolia* (Link) Friis, *Hypotrachyna cirrhata* (Fr.) Divakar, A. Crespo, Sipman, Elix & Lumbsch, *Rhododendron anthopogon* D. Don and *Swertia racemosa* (Griseb.) Wall. ex C.B.Clarke.

Materials and Methods

Collection of plant materials

The studied plants were collected from different parts of Nepal as listed in Table 1. Then the plants were identified and vouchers were deposited in the National Herbarium and Plant Laboratories (KATH) for future reference.

Extraction of plant materials

The extraction of all plant materials were carried out using a cold extraction process as suggested by Harborne (1998) and Nortjie et al. (2022). The specific parts of the studied plant materials were cleaned with tap water, dried under shade, powdered, and macerated with 100% ethanol of 10 times the weight of the plant sample for 72 hours at room temperature with intermittent agitation. Then, the solution was filtered through Whatman grade 1 filter paper and the residues were macerated further 2 times with the recovered alcohol. The filtrates were concentrated by evaporating the solvent using a rotary evaporator under reduced pressure at a

Table 1: Sites and collection date of various studied plant

S.N.	Scientific name	Local name	GPS	Collected from	Collected date	Parts of plant
1.	*Anaphalis triplinervis* (Sims) C.B.Clarke	Buki Phool	Latitude-28.218584° N Longitude-83.811491° E Elevation- 2065 m asl	Panchase, Pokhara	2079/07/03	Flowering twig
2.	*Chlorophytum arundinaceum* Baker	Seto musalee	Latitude-28.70867° N Longitude-80.61883° E Elevation- 158 m asl	Kailali	2079/05/24	Tuber
3.	*Curculigo orchioides* Gaertn.	Kaalo musalee	Latitude-28.8721° N Longitude-80.57564° E Elevation- 222 m asl	Kailali	2079/05/23	Rhizome
4.	*Dolichousnea longissima* (Ach.) Articus	Old man's beard or Methuselah's beard lichen	Latitude-28°6'22.230'' N Longitude-85°21'17.4618'' E Elevation- 3508 m asl	Gosaikunda, Chandanbaari	2079/05/23	Whole part
5.	*Girardinia diversifolia* (Link) Friis	Chalnesisno or Allo	Latitude-28.2318° N Longitude-83.7916° E Elevation- 2517 m asl	Panchase, Pokhara	2079/07/02	Stem and leaf
6.	*Hypotrachyna cirrhata* (Fr.) Divakar, A. Crespo, Sipman, Elix & Lumbschsp	Jhyaau/ lichen	Latitude-28°6'26.31'' N Longitude-85°19'48.018'' E Elevation- 2930 m asl	Gosaikunda, Dhimsa	2079/05/25	Whole part
7.	*Rhododendron anthopogon* D. Don	Sunpati	Latitude-28°5'29.45" N Longitude-85°22'58.100" E Elevation- 3964 m asl	Gosaikunda, Lauribina	2079/05/23	Branch
8.	*Swertia racemosa* (Griseb.) Wall. Ex C.B. Clarke	Chiraito	Latitude-28°5'42.47'' N Longitude-85°22'59.100'' E Elevation- 4150 m asl	Gosaikunda, Lauribina	2079/05/23	Whole part

temperature of 40-45°C. Then the extract was stored in the refrigerator at -4ºC until further use (Seyfe et al., 2017).

Acute toxicity study

This study was performed to confirm the safety profile of these plant extracts according to OECD guidelines for testing of chemicals, test guideline no. 425 (OECD, 2022). Healthy nulliparous and non-pregnant female mice (25-40 grams) were used for all the experiments in the present study. The animals were maintained under standard husbandry conditions in the Animal House Section of Natural Products Research Laboratory, Thapathali, Kathmandu (temperature 25±2°C) in a natural light-dark cycle, and fed with standard rodent diets and water ad libitum. Before experimenting, the animals were randomly selected and grouped into nine groups containing five mice in each group (n=5) with one control group. All groups of mice were fasted overnight, weighed and the doses were calculated based on their body weights.

All extracts were prepared in distilled water, and then were administered orally at the doses of 2000 mg kg^{-1} (group II–IX) body weight of mice in the test groups. The control group (group I) received distilled water only. After administering the extracts, the animals were kept under close clinical observation for mortality, behavioral, neurological and any other abnormalities for 14 days (periodically first 24 hours with special attention given during the first 4 hours). The toxicological effects were observed in terms of mortality and expressed as LD50 (Bruce, 1985). The LD50 for the plant extract showing mortality at 2000 mg kg^{-1} was calculated using the linear regression of the constructed curves, based on the graphical method of Miller and Tainter (1944, as cited in Randhawa, 2009). On the basis of LD50 values, the extracts were classified into different toxicity categories following the criteria prescribed in Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations [UN], 2011).

Ethanol induced gastric ulcer test

Anti-ulcer properties of the plant extracts were evaluated following the protocol outlined by Robert (1979). Healthy, 24-hour fasted albino rats were divided into ten groups of three rats each (n=3), viz., Control group (I), Test groups (group II- IX) and standard group (group X) to which oral administration of only normal saline at 1 ml per 100 gram weight, plant extracts at 500 mg kg^{-1} dissolved in distilled water and sucralfate at 100 mg kg^{-1} were given respectively. After an hour, all ten groups of animals were given ethanol at 1 mL per 200 gram weight orally. The animals were euthanized one hour after ethanol administration using an overdose of ether. Then, a midline incision was made with the scalpel and the stomach was excised along the greater curvature. The gross evaluation of the stomach was done using 10× magnifier lens for scoring the ulceration as the normal colored stomach (0), red coloration (0.5), spot ulcer (1), hemorrhagic streak (1.5), deep ulcers (2) and perforation (3) (Dashputre & Naikwade, 2011; Kulkarni, 1987; Sahoo et al., 2016; Süleyman et al., 2002). Ulcer index (UI) was measured with the given formula below (Deshpande & Balekar, 2018; Roy et al., 2013):

Ulcer index (UI) = Mean sum of the score of ulceration i.e. (the normal colored stomach (0) + red coloration (0.5) + spot ulcer (1) + hemorrhagic streak (1.5) + deep ulcers (2) + perforation (3))

Similarly, the percentage inhibition of ulceration was calculated as below:

$$\%\ inhibiton\ of\ ulceration = \frac{(ulcer\ index\ of\ control\ group - ulcer\ index\ of\ test\ group)}{ulcer\ index\ of\ control\ group} \times 100$$

Charcoal meal test

The experiment was designed by having 10 groups (n=3) with randomly selected albino mice. All animals were fasted for 24 hours with free access to water. Animals in group I served as a standard group with the administration of loperamide (2 mg kg^{-1}). The animals in group X served as negative control and were administered only normal saline at 1 ml per 100 gm mouse, while group II to IX served as

treated groups receiving extracts at 500 mg kg^{-1} orally. After 30 minutes of having given the doses as described above, intestinal motility was assessed by orally administrating semisolid charcoal meal (0.3 mL per mouse) consisting of 10% charcoal and 5% gum acacia dissolved in distilled water. The animals were sacrificed 30 minutes later. The abdomen was opened and the entire small intestine starting from the pyloric end was removed and measured. The distance traveled by charcoal in the small intestine was also measured and expressed as a peristaltic index (Fokam Tagne et al., 2019; René et al., 2015).

$$PI = \frac{DCCM}{TLSI} \times 100$$

Where PI= Peristaltic index, DCCM= Distance traveled by charcoal, and TLSI= Total length of the small intestine

The gastrointestinal motility inhibition was determined by the following formula:

$$I\% = \frac{(PIc - PIt)}{PIc} \times 100$$

Where, PIc = Peristaltic Index in the control group; PIt = Peristaltic Index in the test group.

Statistical analysis

Data were presented as mean±SEM in Microsoft Excel 21. One-way ANOVA at 0.05 level of significance (α) followed by a post hoc test (Dunnett's) was done using IBM SPSS (Statistical Package for Social Sciences) version 26, at 95 % confidence level for multiple comparisons of the mean differences and responses of different plant extracts with the control group and standard group.

Results and Discussion

Acute toxicity test

The acute toxicity study did not show any morbidity, mortality, toxicity signs and symptoms at 2000 mg kg^{-1} in all tested extracts except in *Hypotrachyna cirrhata*. Since more than three mice survived in oral toxicity test, it signified that the LD50 values of the seven plant extracts were greater than 2000 mg kg^{-1} body weight. However, the ethanol extract of *H. cirrhata* showed LD50 of 1984 mg kg^{-1} body weight, hence, was classified as "harmful if swallowed" as per GHS criteria for acute toxicity as shown in Table 2. Our results for non-toxicity of these seven plants are in agreement with the researches done in different corners of the world (Chen et al., 2011; Dobrescu et al., 1993; Frisvad et al., 2018; Mori et al., 2016; Ramchandani et al., 2014; Singh & Bedi, 2018).

Anti-diarrheal test

Among the eight extracts tested, *the Hypotrachyna cirrhata* extract showed the highest inhibition of gastrointestinal motility (29.5%) whereas the *Rhododendron anthopogon* extract showed no inhibition of charcoal movement as shown in Figure 1.

The extract of *Hypotrachyna cirrhata, Swertia racemosa* and the standard group (loperamide) were found to show significant inhibition of gastrointestinal mobility with a p-values of 0.004, 0.037 and <0.001 respectively at 5% level of

Table 2: Result of oral acute toxicity test of ethanol extracts of eight medicinal plants tested in mice

S.N.	Scientific Name	LD50 (mg kg^{-1})	Category as given by GHS (GHS, 2018)
1	*Anaphalis triplinervis*	>2000	5 (may be harmful if swallowed)
2	*Chlorophytum arundinaceuum*	>2000	5 (may be harmful if swallowed)
3	*Curculigo orchioides*	>2000	5 (may be harmful if swallowed)
4	*Dolichousnea longissima*	>2000	5 (may be harmful if swallowed)
5	*Girardinia diversifolia*	>2000	5 (may be harmful if swallowed)
6	*Hypotrachyna cirrhata*	1984	4 (harmful if swallowed)
7	*Rhododendron anthopogon*	>2000	5 (may be harmful if swallowed)
8	*Swertia racemosa*	>2000	5 (may be harmful if swallowed)

significance. It means that the ethanolic extract of *H. cirrhata and S. racemosa* have some anti-diarrheal effects but its effects are comparatively lesser than those of standard drug i.e. loperamide. The remaining seven extracts, were found to have no anti-diarrheal effects in mice as shown in Table 3.

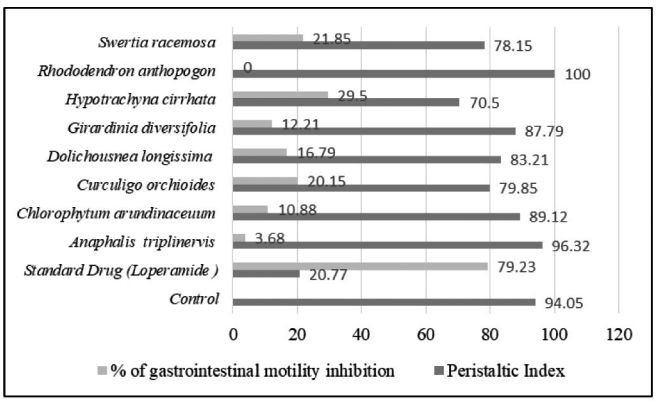

Figure 1: Anti-diarrhoeal activity of ethanol extracts of medicinal plants tested by charcoal movement in mice

Anti- ulcer test

Among the eight extracts, extract of *Hypotrachyna cirrhata* showed the highest capacity of gastric protection (97.82%) while that of *Chlorophytum arundicaceuum* showed no gastric protection capacity in the rat. In our study, *H. cirrhata, Dolichousnea longissima, Rhododendron anthopogon, Curculigo orchioides* and *Girardinia diversifolia* extracts were found to have greater gastric protection than the standard drug i.e. sucralfate as shown in Table 4 and Figure 2. *H. cirrhata* and *D. longissima,* both being lichen, could show anti-ulcer effects as mentioned by Nayaka & Haridas (2020) and Rethinavelu et al. (2023). These plants may have shown anti-gastric ulceration by protecting mucosal barriers of the stomach as cytoprotective agents in peptic ulcer management (Sharifi-Rad et al., 2018).

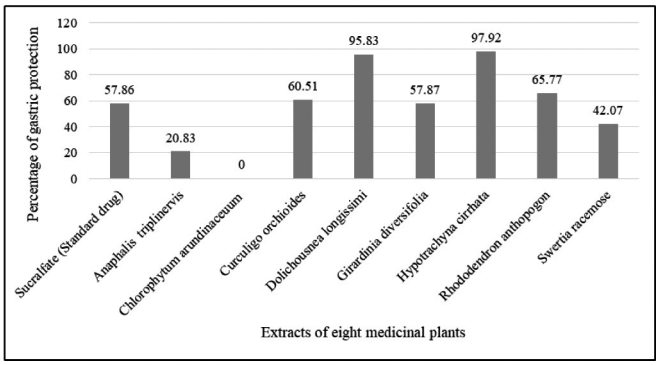

Figure 2: Result of medicinal plants showing anti-ulcer activity in rats

Table 3: Result of ANOVA and post hoc Dunnett test (2-sided) for percentage of inhibition of gastrointestinal motility

S.N.	Treatments	Percentage of inhibition of gastrointestinal motility (Mean±SE)	*p*-value	Inference at 95% confidence interval
1	*Anaphalis triplinervis* group	3.70±3.70	0.998	Non-significant
2	*Chlorophytum. arundinaceuum* group	10.27±6.62	0.624	Non-significant
3	*Curculigo orchioides* group	20.16±0.69	0.062	Non-significant
4	*Dolichousnea longissima* group	10.39±3.15	0.612	Non-significant
5	*Girardinia diversifolia* group	12.40±12.40	0.426	Non-significant
6	*Hypotrachyna cirrhata* group	29.52±1.98	0.004	Significant
7	*Rhododendron anthopogon* group	0.00±0.00	1.000	Non-significant
8	*Swertia racemosa* group	21.92±5.07	0.037	Significant
9	Standard drug (Loperamide)	79.26±0.61	<.001	Very Significant

Table 4: Result of ulcer index and percentage of gastric protection of various plant extracts

S.N.	Treatments	Dose (mg kg^{-1})	Mean Ulcer Index ± SEM
1	Sucralfate (Standard drug)	100	2.67±0.67
2	*Anaphalis triplinervis*	500	5.01±0.96
3	*Chlorophytum arundinaceuum*	500	6.33±0.55
4	*Curculigo orchioides*	500	2.50±1.44
5	*Dolichousnea longissima*	500	0.27±0.19
6	*Girardinia diversifolia*	500	2.67±1.54
7	*Hypotrachyna cirrhata*	500	0.13±0.10
8	*Rhododendron anthopogon*	500	2.17±0.68
9	*Swertia racemosa*	500	3.67±1.35

Conclusion

A total of eight species of medicinal herbs were collected from different parts of Nepal. The LD50 of all tested extracts except that of *Hypotrachyna cirrhata* can be considered relatively safe on acute exposure. *H. cirrhata* ethanol extract was found to show GHS Category 4 toxicity so caution should be taken while using this extract. The ethanol extract of *H. cirrhata* showed the highest inhibition of charcoal movement in the small intestine showing antidiarrheal effects in mice as well as the highest gastric protection ability in rats. Similarly, the ethanol extract of *Swertia racemosa* showed slightly significant inhibition of gastrointestinal motility. However, the remaining seven medicinal plants showed no antidiarrheal effects in mice. Further, the extracts of *Curculigo orchioides, Dolichousnea longissima, Girardinia diversifolia, H. cirrhata,* and *Rhododendron anthopogon* had strong gastro-protective effects in ethanol-induced gastric ulcer. It is concluded that these medicinal plants showing positive pharmacological activities contain certain bioactive compounds that protect mucosal barriers of the stomach as cytoprotective agents in peptic ulcer management. However, isolation and identification of phytochemicals responsible for pharmacological activity with their purification and characterization are necessary for further detailed study.

Author Contributions

Both authors have contributed equally to bring the manuscript in this form.

Acknowledgements

We are grateful to Mr. Devi Prasad Bhandari, NPRL Chief and all staff of NPRL for providing the necessary facilities to complete the research. We would like to thank the Department of Plant Resources (DPR) for the support to carry this work. We are indebted to National Herbarium and Plant Laboratories (KATH), Lalitpur for identification of the herbarium materials. Our deepest gratitude goes to Ms. Chetana Khanal, Mr. Paras Mani Yadav, Mr. Karma Lal Maharjan, Mr. Dipak Shrestha, Mr. Janak Lama and Ms. Sanu Maya Tamang for the help and support during this work.

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