



Ethnopharmacological properties of *Capsella bursa-pastoris* (L.) Medik and *Hedychium coronarium* J.Koenig

Rajendra Gyawali*, Tirtha Maiya Shrestha, Nikita Karki, Anjana Khanal, Beenu Shrestha, Manisha Neupane, Samjhana Shrestha

Department of Pharmacy, Pharmacognosy and Phytochemistry Research Laboratory
Kathmandu University, IDhulikhel, Kavre, GPO Box. 6250, Nepal

*For correspondence: ragyawali@gmail.com

Abstract: Ethnopharmacological evidence confirms the acceptance of botanicals through scientific validation of indigenous practices. Leaves and roots of *Capsella bursa-pastoris* (L.) Medik, and *Hedychium coronarium* J.Koenig are used as vegetable and medicinal purpose in Nepal. Medicinal plants *C. bursa-pastoris*, and *H. coronarium* were subjected to phytochemical screening and biological evaluation. Methanolic extract of plant powders were evaluated for their medicinal properties regarding their phytochemical profiles. Anti-microbial anti-inflammatory, antioxidant, wound healing and brine shrimp cytotoxic activities were studied by standard methods. Extracts of the plants showed the activity against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. *C. bursa-pastoris* is found to be active against *S. Typhi*. Comparatively less toxic and better anti-inflammatory property was exhibited by *H. coronarium*. The preliminary results are particularly applied among ethnopharmacological practitioners of currently practicing systems of traditional medicine in Nepal. The results will be useful in bioprospecting for clinical application and the development of novel herbal therapeutics or consumer products.

Keywords: *Capsella bursa-pastoris* (L.) Medik, *Hedychium coronarium* J.Koenig, Phytochemicals, Ethnopharmacology, Formulation



INTRODUCTION

Multi spectrum therapeutic activities of herbs has been also reported by previous studies [4, 5]. Ethnopharmacological studies demonstrated the scientific validation of traditionally used herbs in the treatment of wounds and also have shown the high level of antioxidant property [6]. Medicinal plants have always played a pivotal role as a source of drug molecules. Medicinally important plant secondary metabolites has been reported for the various pharmacological activities conforming the traditional use and their potentiality [7]. Ethnopharmacological evidence confirms the belief on herbs for the treatment and their acceptance in numerous countries. Ethnobotanicals have been introduced to international medicine through validation of traditional practices [8]. Himalayan plants are potential natural sources that possess a variety of secondary metabolites and drawn the attraction of several researchers in identifying the novel compounds responsible for various medicinal properties.

Leaves and roots of *Capsella bursa-pastoris* (L.) Medik, are used as astringent, bleeding, inflammation, cardio vascular diseases, blood pressure and vegetable in Nepal [9]. Several classes of secondary metabolites such as flavonoids [10,11] alkaloids [12], glucosinolates [13] and saponins [14] are reported from the plant. Plant exhibited several activities such as antioxidant, anticancer, hepato-protective, sedative, antimicrobial, anti-inflammatory etc during the study [15]. The methanolic and aqueous extracts which contained many flavonoids are reported for antioxidant activity [16, 17]. Significant anti-inflammatory activity indicated by the decreased levels of nitric oxide (NO), cytokines, and prostaglandin E2 in lipopolysaccharide-stimulated murine macrophages [18]. Similarly, the seeds of *Hedychium coronarium* J.Koenig are carminative and stomachic while root is antirheumatic, analgesic, fever, excitant and antioxidant tonic [19]. The major components of the rhizome essential oil are 1,8-cineole (41.42%, 37.44%), β -pinene (10.39%, 17.4%) and α -terpineol (8.8%, 6.7%). Due to high content of 1,8-cineol it is considered to have anti-inflammatory property [20]. Fresh sample and dried samples of this plant showed better activity against *Trichoderma sp.*, *Candida albicans*, *Bacillus subtilis* and *Pseudomonas aeruginosa* [21]. Recent finding suggests that, the rhizome is used in treatment of diabetes, cold, body aches, headache, lancinating pain, contusion, inflammation, immune booster and rheumatic pain [20,21,22].

The purpose of this study was to screen the phytochemicals and evaluate the antioxidant, antimicrobial, wound healing, cytotoxic and anti-inflammatory activities of methanolic extract of *H. coronarium* J.Koenig and *C. bursa-pastoris* (L.) Medik.

MATERIALS AND METHODS



Collection of plants and extraction: The roots of *H. coronarium* J.Koenig and whole plant of *C. bursa-pastoris* (L.) Medik were collected during 2017 April from Kavre and Bhaktapur districts of Nepal. They were washed, dried under shade and powdered. The extraction process was optimized in chloroform, petroleum ether and methanol in different proportion to extract in hot and cold condition. Final extraction was carried out in optimized solvent ratio of 1:15 powder:solvent using 80% methanol by cold extraction method in horizontal shaker for one week, which was evaporated in rotavapor after the filtration.

Phytochemical screening: Qualitative phytochemical analyses of alkaloid, tannins, flavonoid, coumarin and reducing sugar in both the plants were performed by following the protocol [23].

Animals: Albino rats (150-200g) and Swiss albino mice (22-30g) of either sex were brought from department of plant resources, ministry of forest and environment, Nepal. They were kept in room temperature ($25 \pm 2^{\circ}\text{C}$) in light and dark cycle of 12. They were supplied standard diet and water ad libitum. The experimental protocol was in accordance with the standard practice of animal handling as accepted internationally [24].

Brine shrimp lethality assay: Shrimp eggs were added into the freshly prepared artificial sea water in beaker and incubated for 48h to hatch as nauplii. Stock solution of 1000 ppm of sea water was used to make up 500 ppm, 250 ppm, 100 ppm, 70 ppm, 50 ppm, 25 ppm and 10 ppm solution of herbal extracts. Herbal extract solutions of different concentration were filled in test tubes and 10 brine shrimps were kept in each test tube. Standard of sea water was also used for the positive control. All test tubes with hatched brine shrimps were incubated for 48 hours in 37°C . The number of brine shrimp surviving in each test tube was counted after 48 h. From the data generated lethal dose 50 (LD_{50}) was calculated [24].

Antimicrobial activity of plant extract: Antimicrobial test was performed by cup-plate method on *Salmonella typhi* and *Staphylococcus aureus* which were obtained from Dhulikhel Hospital, Kathmandu University Teaching Hospital, Nepal. A strip of agar was excavated from a petri dish and replaced it with medium containing the extract of potential antimicrobial activity. Standard ciprofloxacin and nitrofurantoin disc were placed in agar surface loaded with microorganism. It was incubated at 37°C for 48 hours and observed for zone of inhibition [25].



Anti-inflammatory: Induced paw edema method with declofenac gel as standard anti-inflammatory agent was used in albino rats. Each dose group consisting 6 mice were given respective drug dose except control. Each rat was marked in their left hind paw up to common level. After 1 hour the hind left paw of the mice was injected with 25 μ l 1% formalin in the sub-plantar region. After 1,2,3,4 and 5 hour of formalin injection, the paw volume was measured and noted using plethysmometer [26].

Wound healing: The ointment of *C. bursa-pastoris* (L.) Medik extract was evaluated on rats in the incision wound models to confirm the folkloric usage of the plant. A wound was created on the dorsal region of each mouse of group by incision process of the predetermined length 10 mm and medicine was applied topically once a day on to each mouse till the wound was completely healed up to the time period of 8 days. The mice were divided into control, standard, test groups for the comparison of percentage of wound healed [27].

Antioxidant Stock solution of 100 μ M DPPH was prepared in methanol. Concentrations of test samples were prepared at 12.5, 25, 50, 100 μ g/ml in methanol. Ascorbic acid was prepared in similar concentrations. Total 2 ml of each extract was treated with 2 ml of 100 μ M DPPH and kept in dark. Similar volume of ascorbic acid was also mixed with same volume of ascorbic acid and kept in dark for 30 minutes in incubator at 37°C. The absorbance was measured at 517 nm by UV spectrophotometer after 30 minutes and % scavenging was calculated by the following equation:

Percentage scavenging = $(A_0 - A_T) / A_0 \times 100\%$.

Where, A₀ = Absorbance of DPPH solution and A_T = Absorbance of test or reference sample. The % scavenging was then plotted against concentration and regression equation was obtained to calculate IC₅₀ values [28].

Analgesic Albino mice were divided into four groups (n=6 in each group) and treated according to previously approved method [29]. Control group mice were administered with 0.2 ml of purified water. Then after one hour acetic acid 0.2 ml was injected intraperitoneally. Herbal extract group of mice were injected with solution of extract 150 mg/kg in 2 in purified water. Then after one hour 0.2 ml of acetic acid was injected. Where as, mice were injected with paracetamol 150 mg/kg included in 2 ml intraperitoneally as a standard. Writhing effect was observed for 20 minutes after the acetic acid was administered. Percentage protection against writhing movement was taken as an index of analgesia and it was calculated as follows:

Percentage of inhibition = $\{W_r(\text{control}) - W_r(\text{test group})\} / W_r(\text{control})$.

Where W_r = mean no. of writhing

Formulation of ointment: Ointment was prepared by fusion method. Simple formulation containing Polyethylene glycol (PEG) 400 and PEG 4000 were used to prepare 50%



ointment of *H. coronarium* J.Koenig extract. Propylene glycol (PG) was used to dissolve extract.

Linearity: For this, 5% extract of *H. coronarium* J.Koenig was dissolved in 10% DMSO and volume make up by methanol. 10% DMSO in methanol was used as blank. Then maximum absorbance was scanned in the range of 0-4 by serial dilution and minimization of range. Wavelength showing maximum absorbance in the range was selected. Solution of 25ppm, 50ppm, 75ppm, 100ppm and 125ppm were used to check linearity. From the linearity graph, regression coefficient and linearity equation were obtained. This equation was used to find drug release in diffusion testing.

Diffusion of ointment: Diffusion cell apparatus was used to check the permeation of the drug through cell membrane. Ointment and base is loaded in the upper portion of the diffusion chamber. Phosphate buffer (pH: 6.8) was used as diffusion fluid which mimic the skin environment. Cellophane membrane was used as the diffusion membrane. Each gasket was loaded with 40 mg of ointment, equivalent to 2 mg of extract. Diffusion cell apparatus was operated in 150 rpm at 36 degree Celsius. Sample was extracted in every 15 minutes and observed up to 1 hour. Sample was obtained with 1 ml syringe. Extracted sample was analyzed in UV spectroscopy. Wavelength obtained from screening test was used to obtain absorbance and linearity equation was used to calculate concentration of extract in the diffusion solution.

Assessment of ointment: Different parameters of ointment was evaluated after the completion of formulation. Quality of ointment, irritancy, spreadibility, diffusion and permeability were evaluated. By applying the ointment on skin, spreadibility was evaluated. For the irritancy test, the ointment was applied to the normal and broken skin of healthy human volunteers.

RESULTS AND DISCUSSION

Sample preparation: Optimization of extracting solvent of medicinal is also important for maximizing the extraction yield of bioactive compounds because these compounds have different characteristics as well as polarities [30]. Plethora of studies showed that the amount of bioactive phytochemical in the extract and their relative antioxidant property is influenced by the nature of solvent used to prepare that extract [31]. Therefore, it is also necessary to determine the solvents for extraction of bioactive compounds. The results showed that extraction solvents significantly affected on yield where alcohol was most suitable solvent followed by chloroform and then hexane for both of these plants. Hot method extraction of *H. coronarium* J.Koenig yielded maximum extract of 0.09 gm out of 1 gm in 1:5 ratio of solvent but this method is not used since heating may sometime degrade various chemical compounds of the plant. Among cold method, 1:15 ratio of 80% methanol yielded 0.07 gm of extract from 1



gm sample. A similar result showed that water and methanol extracts of *Paramignya trimera* had higher extraction yields than acetonitrile, ethyl acetate and hexane extracts [32]. In *C. bursa-pastoris* (L.) Medik alkaloids, tannins and flavonoid classes were present. In *H. coronarium* J.Koenig reducing sugar, lead and coumarin was found to be present.

Antimicrobial property: Medicinal plants constitute an effective source of antimicrobial natural products [2,33]. The extract of *H. coronarium* J.Koenig is found to be active against *S. typhi* by 15% as compared to Ciprofloxacin 500mg disc and 34.14% as compared to Nitrofurantoin 500 mg disc. *H. coronarium* J.Koenig was also found active against *S. aureus* by 53.83% as compared to standard ciprofloxacin 500 mg disc and 38% as compared to nitrofurantoin 500mg disc. *C. bursa-pastoris* (L.) Medik is found to be active against *S. typhi* by 24.96% as compared to Ciprofloxacin 500mg disc and 56% as compared to Nitrofurantoin 500 mg disc. *C. bursa-pastoris* extract is found active against several bacterial and yeast. They suggested that effectiveness could be caused by some alkaloids and some flavanoids found in extract [34].

Brine shrimp lethality property: Result of brine shrimp lethality assay on *H. coronarium* J.Koenig showed that LC50 is approximately 99.01ppm. Thus, it is found to be less toxic. Result of brine shrimp lethality assay on *C. bursa-pastoris* (L.) Medik showed that LC50 is found to be approximately 43.93ppm. Thus, it is found to be toxic in comparison to *H. coronarium* J.Koenig. Our finding on *C. bursa-pastoris* (L.) Medik is in agreement with previous report, which has been reported to exhibit low toxicity in mice. LD50 values reported are 1.5 g/kg body weight (mice, intraperitoneal injection) and 31.5 g/kg bodyweight (mice, subcutaneous injection) [35]. Brine shrimp lethality assay is used as an indicator for general toxicity and also as a guide for the detection of antitumor agent [24].

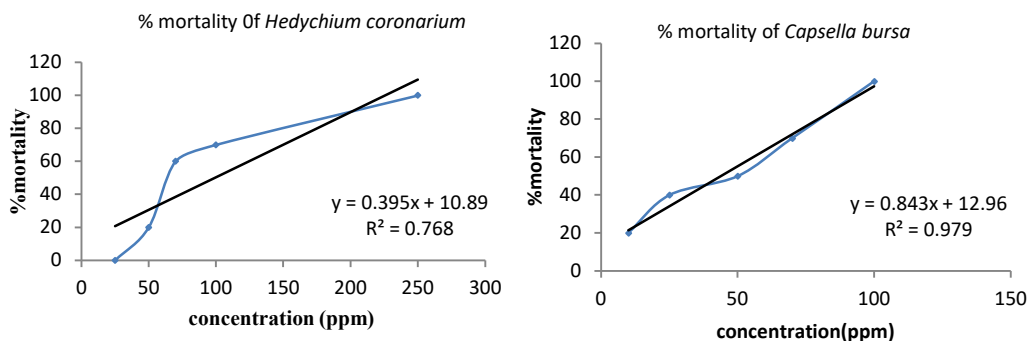


Figure 1: Toxic effect of *H. coronarium* J.Koenig and *C. bursa-pastoris* (L.) Medik in brine shrimp test.



Anti-inflammatory property: In the present study the attempt has been also focused to evaluate the anti-inflammatory activity of extracts using formalin induced paw-oedema in rats as a model. Percentage of inhibition of inflammation at the end of four hour was found to be 56.52 % in standard declofenac applied mice, 40% in *H. coronarium* J.Koenig ointment, 35% in *C. bursa-pastoris* (L.) Medik, 29.41% in ointment base and 25% in control group of mice. Both the chloroform and methanol extracts showed significant elongation of tail flick time and inhibition of paw edema at 400 mg/kg body weight justifies the present finding [22]. Though this plant possesses the anti-inflammatory property but it is equally important to develop products or any drugs in low side effects [36].

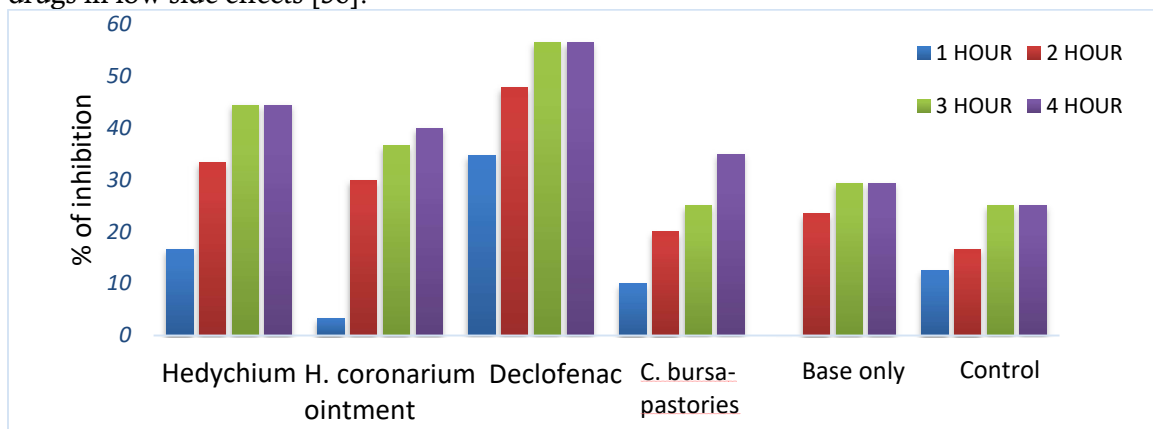


Figure 2: Anti-inflammatory property of *C. bursa-pastoris* (L.) Medik, *H. coronarium* J.Koenig, 5% ointment of *H. coronarium* extract and its base compared to control in albino rats.

Wound healing: The wound area changes induced by the extract were assessed and compared with reference drug and control group. Area of the wound was measured up to 7 days post surgery in all groups. Figure 3 shows the measured values of the closure progression of non-infected wound in different groups. Standard drug showed maximum wound healing property as compared to extract and control group. After application of 5% ointment in rats, wound healing property of the plant was found to be 79% by end of day 7. We observed that the topical application of this formulation enhances cutaneous healing. Wound healing or wound repair is the body's natural process of regenerating dermal and epidermal tissue [37]. The process of wound healing occurs in four phases: (i) coagulation, which prevents blood loss, (ii) inflammation and debridement of wound repair (iii) including cellular proliferation, and (iv) tissue remodeling and collagen deposition [38].

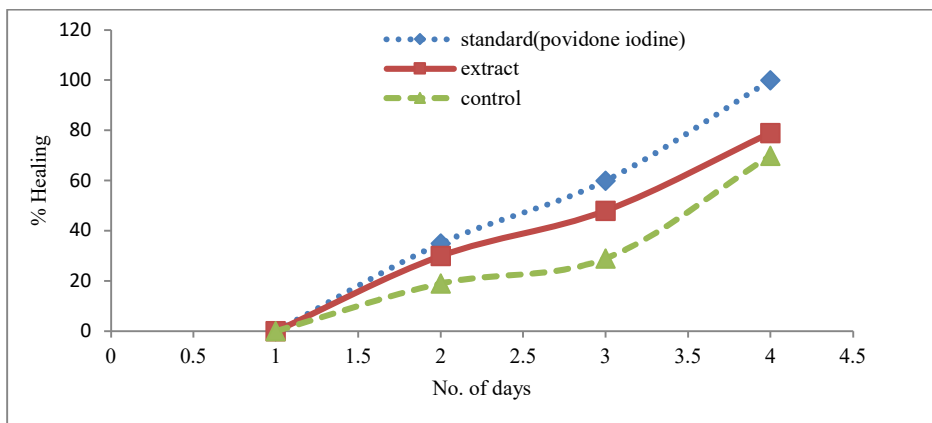


Figure 3: Wound healing property of *C. bursa-pastoris* (L.) Medik extract against standard povidone iodine and control groups of albino rats

Anti-oxidant property: The percentage of inhibition is found to be maximum for ascorbic acid, followed by *C. bursa-pastoris* (L.) Medik and then for *H. coronarium* J.Koenig. In lower concentration *C. bursa-pastoris* (L.) Medik is found to have more anti-oxidative property than ascorbic acid. The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [39,40].

Table 1: Inhibition percentage of oxidation of DPPH by *C. bursa-pastoris* (L.) Medik and *H. coronarium* J.Koenig

Concentration (µg/ml)	% of Inhibition		
	Ascorbic acid	<i>C. bursa-pastoris</i> (L.) Medik	<i>H. coronarium</i> J.Koenig
0.001	46.73	44.06	37.84
0.01	57.83	48.94	44.50
0.1	65.59	63.33	46.72
1	70.03	64.44	50.05

Analgesic property: In the present study the attempt has been focused to evaluate the analgesic property of *C. bursa-pastoris* (L.) Medik against acetic acid induced writhing response in mice. Percentage of inhibition of pain induced by acetic acid is 80% in case of standard paracetamol injection. But *C. bursa-pastoris* (L.) Medik did not showed the analgesic property during this study. Therefore, it is concluded that, this plant may not be a candidate for future to study as analgesic herb with the proper study of its mechanism of action [41].

**Table 2: Number of writhing caused by acetic in albino rats by *C. bursa-pastoris* (L.) Medik extract as compared to standard paracetamol injection.**

S.N	Groups	No of writhing
1.	Control	20
2.	Paracetamol	4
3.	<i>C. Bursa-pastoris</i>	55

Ointment assessment: Assessment of the spreadability, irritancy, and diffusion test of ointment was performed. When the ointment was applied to the normal and broken skin, it showed no irritant effect. The ointment readily spread when applied on the skin topically and rubbed gently. The product well diffused when tested using diffusion test apparatus that drug release in 60 minutes was found to be 78.42%.

Table 3: Diffusion pattern of ointment of *H. coronarium* J.Koenig extract

Time (min)	Drug content(μ g)		% drug release	Cumulative drug release
	In 2 ml	In 7.5 ml		
15	123	461.25	23.05	23.05
30	201	753.75	37.68	43.85
45	44.5 * 5	834.375	41.71	57.9
60	54.5 * 5	1021.87	51.09	78.42

CONCLUSION

Methanol was found as a best solvent for the extraction of *C. bursa-pastoris* (L.) Medik and *H. coronarium* J.Koenig. Phytochemical screening resulted to presence in tannin and flavonoid that might be main reason for the antioxidant property of the extract. *C. bursa-pastoris* (L.) Medik was effective against *S. typhi* but *H. coronarium* J.Koenig showed better anti-inflammatory property. The wound healing activity of the *C. bursa-pastoris* (L.) Medik ointment was found effective at the end of day 7.

ACKNOWLEDGEMENT

Authors would like to thank to University Grants Commission of Nepal for publication support of this paper from institutional research grant. We would like to also acknowledge to KU-NTIC/IRDP for small help during publication. We have no conflict of interest to declare.



CONFLICT OF INTEREST

We have no conflict of interest to declare.

REFERENCES

1. Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul T, Devasagayam A, Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes, *J Clin Biochem Nutr*, 40(3) (2007) 163-173.
2. Adhikary P, K.C. R, Kayastha D, Thapa D, Shrestha R, Shrestha TM, Gyawali R, *In-Vitro* evaluation of antimicrobial and cytotoxic potential of dry rhizome extract of *Astilbe rivulari*, *International Journal of Pharmacognosy and Phytochemical Research*; 4(3) (2012) 122-126.
3. Oladosu IA, Ogundajo AL, Alyalaagbe OO, Emenyonu N., Phytochemical and antituberculosis activity of *Coffea brivipes*, hiern extracts. *Res J Phytochem.*, 5 (2011) 130–135.
4. Bhutkar MA, Bhise SB, Comparison of Antioxidant Activity of Some Antidiabetic Plants, *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2(3) (2011) 982-987.
5. Calixto JB, Cabrini DA, Ferreira J, Campos MM. Kinins in pain and inflammation, *Pain*, 87(2000) 1–5.
6. Süntar IP, Koca U, Akkol EK, Yilmazer D, and Alper M, Assessment of wound healing activity of the aqueous extracts of *Colutea cilicica* Boiss. & Bal. fruits and leaves, *Evid. Based Complement. Alternat. Med.*, vol. 2011, Article ID 2011.758191, 7 pages
7. Alam B, Akter F, Pravin N, Sharmin Pia R, Akter S, Chowdhury J, Sifath E Jahan K, Haque E, Antioxidant, analgesic and anti-inflammatory activities of the methanolic extract of *Piper betle* leaves, *Advanced J Phytomed*, 3(2) (2013)112-25.
8. Gyawali R, Gupta RK, Shrestha S, Joshi R, Paudel PN. Formulation and evaluation of polyherbal cream containing *Cinnamomum zeylanicum* Blume, *Glycyrrhiza glabra* L and *Azadirachta indica* A. Juss extracts to topical use. *Journal of Institute of Science and Technology*, 25(2) (2020) 61-71.
9. Dani RS, Tiwari A. Medicinal weeds in the rice field of Kathmandu valley, Nepal, *Himalayan Biodiversity* 6 (2018)16-26.
10. Kweon MH, Kwak JH, Ra KS, Sung HC, Yang HC, Structural characterization of a flavonoid compound scavenging superoxide anion radical isolated from *Capsella bursa-pastoris*, *J Biochem Mol Biol*, 29 (1966) 423-428.
11. Song N, Xu W, Guan H, Liu X, Wang Y, Nie X, Several flavonoids from *Capsella bursa-pastoris* (L.) Medik, *Asian Journal of Traditional Medicines* 2 (2007) 218-222.
12. Khare CP, Indian medicinal plants, an illustrated dictionary, Springer Science and Business Media, LLC, (2007) 119.



13. Brock A, Herzfeld T, Paschke R, Koch M, Dräger B, Brassicaceae contain nortropane alkaloids. *Phytochemistry*, 67: (2006) 2050-2057.
14. Cole RA, Isothiocyanates, nitriles and thiocyanates as products of autolysis of glucosinolates in Cruciferae, *Phytochemistry*, 15 (1976) 759-762.
15. Marquina JMG, Villa MG, Garriga AB, Fracción saponínica de la "*Capsella bursa pastoris*", *An R Acad Farm*, 21 (1955) 49-60.
16. Ali Esmail, Al-Snafi, The chemical constituents and pharmacological effects of *Capsella bursa pastoris*, *International Journal of Phamacology & Toxicology*, (2016).
17. KubinováR, Spačková V, Svajdlenka E and Lučivjanská K, Antioxidant activity of extracts and HPLC analysis offlavonoids from *Capella bursa-pastoris* (L.) Medik., *Ceska Slov Farm*, 62(4) (2013) 174-176.
18. Grosso C, Vinholes J, Silva LR, de Pinho BG, Gonçalves RF, Valentão P, Jäger AK and Andrade PB, Chemical composition and biological screening of *Capsella bursa-pastoris*, *Brazilian Journal of Pharmacognosy*, 21(4) (2011) 635-644.
19. Choi WJ, Kim SK, Park HK, Sohn UD and Kim W, Anti-inflammatory and anti-superbacterial properties of sulforaphanefrom Shepherd's Purse, *Korean J PhysiolPharmacol* , 18(1) (2014) 33-39.
20. Duke JA and Ayensu ES, *Medicinal Plants of China*, Reference publications Inc, USA (1985).
21. Tailor Chandra Shekhar, Goyal Anju , A comprehensive review on *Hedychium coronarium* J. KOENIG(*Dolanchampa*, *Kapurlachri*), *Int.J.Res.Ayurveda Pharm*, 6(1) (2015) 98-100.
22. Shrotriya S, Ali MS, Saha A, Bachar SC, Islam MS, Anti-inflammatory and analgesic effects of *Hedychium coronarium* Koen, *Pak J Pharma Sci*, 20(1) (2007) 47-51.
23. Evans, W. C., Evans, D., & Trease, G. E. (2009). *Trease and Evans pharmacognosy*. Edinburgh: Saunders/Elsevier.
24. Pasupuleti MK, Molahally SS, Salwaji S. Ethical guidelines, animal profile, various animal models used in periodontal research with alternatives and future perspectives. *J Indian Soc Periodontol*. 20(4)(2016) 360-368.
25. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL, Brine shrimp: A convenient general bioassay for active plant constituents, *Planta Med*, 45 (1982) 31–34.
26. Shahidi BH, Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumonia* and *Bordetella bronchoseptica*, *Asian J Plant Sci*, 3 (2004) 82–86.
27. Winter CA, Risely EA, Nuss CW, Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs, *Proc Soc Experimental Biol Med*, 11 (1962) 544–547.



28. Diwan PV, Tilloo LD, Kulkarni DR, Influence of *Tridax procumbens* on wound healing, *Ind J Med Res*, 75 (1982) 460-464.
29. Yildirim A, Oktay M, Bilaloğlu V, The antioxidant activity of leaves of *Cydonia vulgaris*, *Turkish Journal of Medical Science*, 31 (2001)23-27.
30. Koster R., Anderson M., de Beer E.J, Acetic acid for analgesic screening, *Fed. Proc*, 18 (1959) 412–418.
31. Jaiswal A.K, Rajauria G, Abu-Ghannam N, Gupta S, Effect of Different Solvents on Polyphenolic Content, Antioxidant Capacity and Antibacterial Activity of Irish York Cabbage, *J. Food Biochem*, 36 (2012) 344–358.
32. Kalia K, Sharma K, Singh HP, Singh B, Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of *Potentilla atrosanguinea* Lodd. and quantification of its phenolic constituents by RP-HPLC, *J. Agric. Food Chem*, 56 (2008) 10129–10134.
33. Nguyen VT, Bowyer MC, Vuong QV, van Altena IA, Scarlett CJ, Phytochemicals and antioxidant capacity of Xiao tam phan (*Paramignya trimeria*) root as affected by various solvents and extraction methods, *Ind. Crops Prod*, 67 (2015) 192–200.
34. Acharya SR, Shrestha A, Gautam B, Maharjan S, Gyawali R, Basyal D, Antioxidant and antimicrobial properties of leaves of *Lyonia ovalifolia* (Wallich), *International Journal of Pharmaceutical & Biological Archives*, 5(4) (2014) 76 -81.
35. Hasan RN, Ali MR, Shakier SM, Khudhair MM, Hussin MS, Kadum YA, Mohammed AI and Abbas AA, Antibacterial activity of aqueous and alcoholic extracts of *Capsella bursa* against Selected Pathogenic Bacteria, *American Journal of BioScience*, Vol. 1(1) (2013) 6-10.
36. Jurisson S, Flavonoid substances of *Capsella bursa-pastoris*, *Farmatsiya (Moscow)*, 2.2 (1973) 34-35.
37. Reinke JM, Sorg H, Wound Repair and Regeneration. *Eur Surg Res* 49 (2012) 35-43.
38. Dr.Matadeen Bharti, Evaluation of wound healing activity of *cissus quadrangularis*, *World journal of pharmacy and pharmaceutical sciences*, 3(6) (2014) 822-834.
39. Puratchikody A, Nithya C, Nagalakshmi G, Wound healing activity of *Cyperus rotundus* Linn., *Indian Journal of Pharmaceutical Sciences*, 68(1) (2006) 97– 101.
40. Young IS, Woodside JV, Antioxidants in health and disease, *J. Clin. Pathol*, 54 (2001) 176-186.
41. Cao G, Sofic ER, Prior RL, Antioxidant capacity of tea and common vegetables, *J. Agric Food Chem.*, 44 (1996) 3426-3431.