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Pharmacognostical comparison and standardization of the wild and cultivated rhizomes of *Rheum australe* (Padamchal) of Nepal

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Abstract
The aims of present work are quality comparison and standardization of the rhizomes of *Rheum australe* (synonym *R. emodi*) of Nepal. The rhizomes of two wild and one cultivated varieties were collected from the Himalayan region. Macroscopic and microscopic analyses, phytochemical screening, fluorescence analysis, physicochemical analysis and determination of nutritive values in the rhizome samples were carried out. Calcium oxalate crystals and starch grains were found to be deposited in the samples. Terpenoids, fatty acids, flavonoids, anthraquinones, polyphenols, glycosides, carbohydrates and saponins were the major phytochemical constituents. Elemental analysis revealed the presence of K, Na, Fe and Mn, and absence of Cd, Cr and Cu. Values of total fat (1.78-4.22%), carbohydrate (24.95-30.40%) and flavonoid (2.77-2.97 g QE/100 g DM) contents in both wild and cultivated samples were nearly close, while a low phenolic content (102.82 mg GAE/100 g DM) was estimated in the rhizomes of cultivated variety compared to that of wild varieties (738.00-766.52 mg GAE/100 g DM).

Keywords: Ash values, Elemental analysis, Fluorescence analysis, Nutritive values, Phytochemicals, Total flavonoid content, Total phenolic content.

1. Introduction
Rheum (family Polygonaceae) has been using for more than two thousand years in the traditional medical systems and is known as the "Lord" or "King of herbs" [1]. It was first mentioned in the Chinese herbal Pen-King (about 2700 B.C.) as a purgative and stomachic; and since then it has been cultivated and used in domestic purposes [2, 3]. Anthraquinones, anthrones, stilbenes, flavonoids, acylglucosides, pyrones, etc. have been isolated from the genus *Rheum* and these constituents have exhibited several pharmacological activities [4].
R. australe D. Don (synonym *R. emodi* Wall. ex Meisn.) is a perennial stout herb that naturally grown on moraines and crevices near streams in the alpine and sub-alpine zones of Himalaya above 3200 m altitude (Fig 1). It can be cultivated in well drained, porous, humus-rich soil above 1800 m [5, 6]. In Nepal, the rhizomes and petioles of *R. australe* (called as Padamchal) are used as laxative, anthelmintic, and to treat infection, sore throat, skin disease, gastritis, liver disease, gout, dislocated bones and pregnancy-induced hypertension etc [7]. They are chewed raw or used to make pickle. Immense importance of *R. australe* is described in several review articles [8, 10].

Fig 1: *R. australe* in its natural habitat

Herbal formulation contributes significantly in improvement of human health. Effectiveness of the traditional medicine should be controlled with reproducible standards and prevention of adulteration. This can be achieved through standardization of quality, quantity and therapeutic effect of the herbs used which indeed rely on phytochemical constituents and proportions. Recently, we have reported antibacterial activity, cytotoxicity and antioxidant capacity of the rhizome extracts of *R. australe* collected from the Manaslu Conservation Area, Nepal [11]. From the plant material, we have isolated two anthraquinones (chrysophanol and emodin) that can act as the stimulant and laxative [12]. In order to quality standardization of *R. australe* grown in Nepal, herein we compare phytochemicals present in wild and cultivated species collected from different parts in the country.

2. Materials and Methods

2.1 Plant materials

Fresh and healthy rhizomes of *R. australe* were collected from the Manaslu Conservation Area, Gorkha District, Western Nepal (collection date: 2012 June, altitude: 3200 m, variety: wild, code: RAM); Sagarmatha National Park, Solukhumbu District, Eastern Nepal (collection date: 2013 July, altitude: 3600-3800 m, variety: wild, code: RAS) and Rampol Village Development Committee, Taplejung District, Eastern Nepal (collection date: 2012 May, altitude: 2200 m, variety: cultivated, code: RAR). The plants were authenticated from the National Herbarium and Research Laboratory, Godawari, Lalitpur, Nepal. The collected rhizomes were dried in shade for several weeks, coarsely ground and used for the analyses.

2.2 Macroscopic and microscopic analysis

WHO guidelines were adopted for the macroscopic and microscopic analyses of the rhizomes [13].

2.3 Phytochemical screening

Using parallel Soxhlet extractors, the plant materials (100 g each) were successively extracted with different solvents (hexane, 500 ml, 16 h; ethyl acetate, 500 ml, 24 h; methanol, 500 ml, 28 h and water, 500 ml, 24 h). After complete evaporation of the solvents, corresponding hexane, ethyl acetate, methanolic and aqueous extracts were obtained, and were immediately used for the phytochemical screening tests [14].

2.4 Fluorescence analysis

The fluorescence behavior of coarsely ground rhizome powders was studied by treating with various reagents and inspection under visible and ultraviolet lights [15, 16].

2.5 Determination of ash

The contents of total ash, acid-insoluble ash and water-soluble ash in the dried rhizomes were determined following reported methods [17].

2.6 Elemental analysis

Total ash obtained from 1 g of plant sample was digested with nitric acid and stock sample solution prepared (total volume 50 ml) was used for estimation of trace elements by using atomic absorption spectrometer (AA5-200 Series, Agilent Technologies) or flame photometer (Chemito 102, Toshniwal Instrument) after appropriate dilution. A detail procedure is described in our previous publication [18].

2.7 Estimation of crude fat

The crude fat content in the plant material was estimated by AOAC method [19].

2.8 Estimation of protein

Kjeldahl's method was employed to determine the protein content [20]. Briefly, the plant material (0.5 g) was treated with a digestion mixture of $CuSO_4/K_2SO_4$, 1:18 (0.25 g) and conc. H_2SO_4 (7 ml) over a sand bath (~350 ˚C) for 7 h. After cooling, 40% NaOH solution (50 ml) was added and distilled. The distillate containing ammonia was trapped in 2% boric acid solution (10 ml) and titration was carried out with 0.01 N H_2SO_4 to determine percentage of protein.

2.9 Estimation of carbohydrate

Total carbohydrate was estimated by the Phenol sulfuric acid method [21, 22].100 mg of the sample was hydrolyzed with 2.5 N HCl (5 ml) over boiling water bath for 3 h followed by neutralization with solid Na_2CO_3. The supernatant was collected and the volume was made to 100 ml using distilled water. The spectrophotometric analysis was carried out at 490 nm after addition of 5% aq. phenol and conc. H_2SO_4.

2.10 Total phenolic and flavonoid contents

Soxhlet extraction of the rhizome powder (100 g) using 80% EtOH (500 ml) for 8 h followed by concentration yielded the plant extract, which was used to determine total phenolic and flavonoid contents.

The Folin-Ciocalteu method was used to determine total phenolic content (TPC) in the rhizomes of *R. australe* [23, 24]. Each extract (5 mg) was diluted to 100 ml with distilled water (concentration 50 µg/ml). The solution (1 ml) was mixed with 0.5 ml of Folin-Ciocalteu's phenol reagent (Merck Specialities Pvt. Ltd., Mumbai) and 2 ml of aq. Na_2CO_3 solution (75 g/l). After keeping under dark for 1 h, absorbance was measured at 760 nm using T80+ UV-VIS spectrophotometer (PG Instrument Ltd.). Gallic acid (Sisco Research Laboratories Pvt. Ltd., Mumbai) solutions (10-150 µg/ml in distilled water) were used to produce the calibration curve. The TPC in the sample was calculated and expressed in mg of gallic acid equivalents (GAE)/100 g of dried mass (DM).

Total flavonoid content (TFC) in the rhizomes of *R. australe* was estimated by the Aluminium chloride method [25, 26]. Each unhydrolyzed extract obtained above (100 mg) was diluted to 10 ml using methanol (concentration 10 mg/ml). The solution (0.5 ml) was mixed with methanol (1.5 ml), 1% methanolic $AlCl_3$ (0.1 ml), 1M KOAc in methanol (0.1 ml) and distilled water (2.8 ml), and the absorbance was measured at 415 nm. Quercetin (Sigma-Aldrich) solutions (25-200 µg/ml in methanol) were used to produce the calibration curve. The TFC in the sample was calculated and expressed in g of quercetin equivalents (QE)/100 g of DM. Next, TFC was estimated after hydrolysis of the plant extract. For this, 100 mg of each unhydrolyzed extract was refluxed with 2N aq. HCl (50 ml) for 1 h. The mixture was extracted with Et_2O (100 ml × 3) and concentrated to obtain residue. Thus obtained residue was diluted to 10 ml with methanol and used for spectrophotometry as described above.

3. Results and Discussion

Young rhizome of *R. australe* was cylindrical, long and spongy with firm and compact texture. Outer surface was seldom much wrinkled after drying. It shrunk when dried and softened to cut after moistened. White reticulations were

commonly absent, white and red lines being parallel to one another. Continuous rings of star-spots were more scattered on the transverse section. The network of white lines on the outer surface was not seen when deep peeling was carried. The roots were readily distinguished by their long cylindrical shape and were entirely radiated in the transverse section. The rhizome had bitter, astringent taste and aromatic odor. The fleshy mass of rhizome was orange in color when fresh but gradually changed to brown. On microscopic examination, the yellowish brown rhizome powder showed the presence of calcium oxalate crystals, which were found to be scattered in some parenchymatous cells. Cork cells were nearly rectangular, thick walled, abundant parenchymatous, filled with elongated starch grains and vessels with reticulate thickening.

For phytochemical screening; hexane, ethyl acetate, methanolic and aqueous extracts of the rhizomes were prepared and the yields obtained are presented in Table 1. The presence of different groups of natural pigments in the plant extracts was identified by different chemical tests: Dragendorff, sulfuric acid, Liebermann-Burchard, spot, fluorescence, Shinoda, Borntiager, ferric chloride, ammonium hydroxide, basic, Molisch and froth tests for alkaloids, carotenoids, sterols and triterpenes, fatty acids, coumarins, flavonoids, anthraquinones, tannins and polyphenols, glycosides, anthocyanosides and anthracenosides, carbohydrates and saponins, respectively. All the samples of *R. australe* rhizome constituted terpenoids, fatty acids, flavonoids, anthraquinones, polyphenols, glycosides, carbohydrates and saponins, whereas alkaloids, carotenoids, coumarins, anthocyanosides and anthracenosides were not detected [9, 27].

Table 1: Extracts' yield from the rhizomes of *R. australe*

Extract	RAM (Yield %)	RAS (Yield %)	RAR (Yield %)
Hexane	2.27	1.24	0.98
Ethyl acetate	4.04	5.60	3.90
Methanolic	18.96	16.30	18.20
Aqueous	40.92	42.21	30.61

The information obtained from fluorescence analysis is useful to evaluate genuineness of the plant material for its medicinal efficacies as it provides ideas about the chemical constituents. Table 2 summarizes the results of fluorescence analysis of the rhizome powder of *R. australe*.

Analyses of various physicochemical parameters of the rhizomes of *R. australe* are summarized in Table 3.

Table 2: Fluorescence analysis of the rhizome powder

Reagent used	Coloration with RAM under the light source			Coloration with RAS under the light source			Coloration with RAR under the light source		
	Visible	UV (365 nm)	UV (254 nm)	Visible	UV (365 nm)	UV (254 nm)	Visible	UV (365 nm)	UV (254 nm)
Distilled water	Br	Bk	Br-Bk	Br	Bk	Br	Y-Br	Bk	Br
5% aq. I_2	Y-Bk	Bk	G-Bk	Y-Br	Bk	G-Bk	Y-O	Br	Y-G
5% aq. KOH	R-Bk	Bk	Bk	R-Bk	Bk	Bk	R	Bk	Bk
5% aq. $FeCl_3$	Br	Bk	Bk	Br	Bk	Bk	Bk	Bk	Bk
Acetone	G-Y	Y-Br	G-Y	G-Y	Y-Br	G-Y	Y	Y-Br	G-Y
Conc. HCl	Br	Br-Bk	Bk	Br	Br-Bk	G-Bk	Br	Br-Bk	G-Bk
Conc. HNO_3	G-O	Br	G-Bk	Br-O	Br	G-Bk	G-O	Br	G-Bk
Acetic acid	Br	Bk	Bk	G-Y	Bk	Bk	Br	Bk	Bk
5% aq. picric acid	Br	Bk	Bk	Br	Bk	Bk	Br	Bk	Bk
5% aq. $K_2Cr_2O_7$	R-Br	Br	Bk	R-Br	Br	Bk	R-Br	Br	Bk

Bk = Black, Br = Brown, G = Green, O = Orange, R = Red, Y = Yellow

Table 3: Physicochemical analyses of the rhizomes of *R. australe*

Physicochemical parameter	RAM	RAS	RAR
Total Ash (%)	6.91	6.81	7.02
Acid insoluble Ash (%)	1.55	1.49	1.05
Water soluble Ash (%)	5.20	4.83	4.91
K (mg/l, mg/g DM)	499.20, 24.96	716.80, 35.84	768.00, 38.40
Na (mg/l, mg/g DM)	46.40, 2.32	36.00, 1.80	30.40, 1.52
Fe (mg/l, mg/g DM)	17.83, 0.89	15.34, 0.77	8.51, 0.43
Cd (mg/l)	-	-	0.001
Cr (mg/l)	-	-	-
Cu (mg/l)	-	-	-
Mn (mg/l, mg/g DM)	1.01, 0.05	3.85, 0.19	1.32, 0.07
Total crude fat (%)	4.22	3.72	1.78
Total crude protein (%)	-	-	-
Total carbohydrate (%)	24.95	25.83	30.40
TPC (mg GAE/100 g DM)	766.52	738.00	102.82
TFC in unhydrolyzed extract (g QE/100 g DM)	2.19	2.25	2.35
TFC after hydrolysis (g QE/100 g DM)	2.77	2.79	2.97

Ash value is important to assure the quality and purity of the crude plant materials. It is used to know the presence of various impurities such as carbonate, oxalate and silicate. The acid insoluble ash mainly contains silica indicating contamination with earthy materials and the value of water soluble ash is used to estimate the presence of water soluble inorganic salts. Values for water soluble ash were found to be higher than acid insoluble ash, which indicated that the

rhizomes are suited for the therapeutic effects [28].

The amount of elements (K, Na, Fe, Cd, Cr, Cu and Mn) present in the rhizome powder was estimated. Comparing to the literature [29], the rhizomes of *R. australe* of Nepal constituted higher concentration of K, Na, Fe and Mn, and a lowered concentration of Cu. Potassium is helpful in reducing hypertension and is found in higher concentration in the cultivated rhizome. Sodium helps in transportation of amino acids and glucose in the blood, and iron helps in blood purification and prevents anemia. The concentration of these elements was higher in the wild rhizomes. Manganese helps in metabolisms, and it was found three folds excess in the rhizomes (RAS) collected from a very high altitude in the Mount Everest region comparing to the other samples. Cadmium, chromium and copper were found to be absent in both wild and cultivated rhizome samples.

As can be seen in Table 3, the rhizome of *R. australe* is a moderately good source of carbohydrate (24.95-30.40%) and fat (1.78-4.22%) but not protein.

It has been reported that *R. australe* rhizome contains phenolic acids (gallic acid, coumaric acid, ferulic acid and chlorogenic acids) and flavonoids (myricetin, quercetin and catechin) that responsible for the antioxidant potentiality of the plant material [30-32]. We have estimated TPC value (738.00-766.52 mg GAE/100 g DM) and TFC value (2.77-2.79 g QE/100 g DM in hydrolyzed extract) in the samples of wild variety (RAM and RAS), and the values were comparable to a recent report of Wasim *et al.* from India [28]. In contrast, TPC value (calculated 37.37 mg/100 g DM) and TFC value (calculated 0.088 g/100 g DM) in the rhizomes of *R. australe*, recently reported by Jahan *et al.* from Pakistan using reverse phase high-performance liquid chromatography, were remarkably less perhaps due to investigating of cultivated sample and consideration of individual constituents [33]. In the analysis of cultivated sample (RAR) in our case, TPC value (102.82 mg GAE/100 g DM) was found seven folds reduced, while TFC value (2.97 g QE/100 g DM in hydrolyzed extract) was found slightly greater than that of wild samples (RAM and RAS). Considering a low phenolic content estimated in the cultivated variety, it can be attributed that naturally grown *R. australe* may exhibit a high antioxidant activity. Furthermore, about 20% of the total flavonoids existed in their glycosidic forms. It is noteworthy to mention that habitation and climatic stress (such as; altitude, soil, temperature, precipitation, etc) have always more or less remarkable affect on the production and accumulation of the phytochemical constituents in the plant materials.

4. Conclusion

In conclusion, pharmacognostical comparison and standardization of wild and cultivated varieties of the rhizomes of *R. australe* of Nepal were carried out. Depending on the phenolic content, the rhizome of wild (high TPC) and cultivated (low TPC) varieties can be differentiated. Further comparison of the plant extracts in various bioactivities through different biological assays is now under progress in our laboratory and will be reported in due course.

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6. References

1. Wang A, Yang M, Liu J. Molecular phylogeny, recent radiation and evolution of gross morphology of the Rhubarb genus *Rheum* (Polygonaceae) inferred from chloroplast DNA *trn*L-F sequences. Annals of Botany 2005; 96(3):489–498.
2. Foust CM. Rhubarb: The Wondrous Drug. Princeton University Press, Princeton, New Jersey, 1992.
3. Lloyd JU. Origin and History of All the Pharmacopeial Vegetable Drugs, Chemicals and Prepartions with Bibliography. American Drug Manufacturers Association, 2008.
4. Zheng QX, Wu HF, Guo J, Nan HJ, Chen SL, Yang JS, *et al.* Review of Rhubarbs: Chemistry and pharmacology. Chinese Herbal Medicines 2013; 5(1):9–32.
5. CSIR. The Wealth of India. Vol. IX, Publications and Information Directorate, CSIR, New Delhi, 1972, 3–4.
6. Chatterjee A, Parkashi SC. The Treatise on Indian Medicinal Plants. Vol. I, PID, New Delhi, 1991.
7. Ghimire SK, Sapkota IB, Oli BR, Parajuli RR. Non-timber Forest Products of Nepal Himalaya. WWF Nepal, Kathmandu, Nepal 2008, 116–117.
8. Rokaya MB, Münzbergová Z, Timisina B, Bhattarai KR. *Rheum australe* D. Don: A review of its botany, ethnobotany, phytochemistry and pharmacology. Journal of Ethnopharmacology 2012; 141:761–774.
9. Nazir S, Sharma M, Saxena M, Abrar M, Ajaz M. *Rheum emodi*: Phytochemistry, bioactive compounds and their biological activity. International Journal of Phytopharmacology 2013; 4(4):272–276. (www.onlineijp.com/download.php?id=214&f=214_272-276.pdf).
10. Bilal S, Mir MR, Parrah JD, Tiwari BK, Tripathi V, Singh P, *et al.* Rhubarb: The wondrous drug. A review. International Journal of Pharmacy and Biological Sciences 2013; 3(3):228–233.
11. Gupta RK, Bajracharya GB, Jha RN. Antibacterial activity, cytotoxicity, antioxidant capacity and phytochemicals of *Rheum australe* rhizomes of Nepal. Journal of Pharmacognosy and Phytochemistry 2014; 2(6):125–128.
12. Huang KC. The Pharmacology of Chinese Herbs. Edn 2, CRC Press, Boca Raton, 1999.
13. WHO. Quality Control Methods for Herbal Materials. World Health Organization, Geneva, 2011, 11–21.
14. Ciulei I. Methods for Studying Vegetables Drugs. Chemical Industries Branch, Division of Industrial Operations, UNIDO, Bucharest, Romania, 1982.
15. Chase CR Jr, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Journal of the American Pharmaceutical Association 1949; 38(6):324–331.
16. Kokoski CJ, Kokoski RJ, Slama FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. Journal of the American Pharmaceutical Association 1958; 47(10):715–717.
17. WHO. Quality Control Methods for Herbal Materials. World Health Organization, Geneva, 2011, 29.
18. Bajracharya GB, Bhetuwal BR, K.C. R. An unexplored, wild and consumable vegetable *Allium prattii*: Nutritional value, cytotoxicity and antibacterial potentiality. Journal of Nepal Chemical Society 2013; 31(1):1–8.
19. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists (Ed. Helrich K). Edn 15,

Virginia, USA, 1990, 79.
20. Horneck DA, Miller RO. Determination of total nitrogen in plant tissue. In: Handbook of Reference Methods for Plant Analysis (Ed. Kalra YP). CRC Press, Boca Raton, 1998, 75–83.
21. Hodge JE, Hofreiter BT. Determination of total carbohydrate by anthrone method. In: Carbohydrate Chemistry (Eds. Whistler RL, Be Miller JN). Academic Press, New York, 1962, 17–22.
22. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 1956; 28(3):350–356.
23. Folin O, Ciocalteu V. On tyrosine and tryptophan determination in protein. The Journal of Biological Chemistry 1927; 73:627–650.
24. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 1999; 299:152–178.
25. Kalita P, Tapan BK, Pal TK, Kalita R. Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. Journal of Drug Delivery and Therapeutics 2013; 3(4):33–37. [http://jddtonline.info/index.php/jddt/article/view/546].
26. Tuladhar SM, Bajracharya GB, Pokharel D, Giri R. The potentially anticarcinogenic flavonoids in vegetables, fruits and spices. Nepal Journal of Science and Technology 2000; 2:17–26.
27. Wani SA, Ashfaq M, Shah KW, Singh D. Phytochemical screening of methanolic extracts of *Podophyllum hexandrum* Royle and *Rheum emodi* Wall. Journal of Current Chemical and Pharmaceutical Sciences 2012; 2(2):125–128 (http://www.sadgurupublications.com).
28. Wasim A, Mohd. M, Sayeed A, SMA Z. Current strategy for research on quality identification of *Rheum emodi* Wall. rhizome. International Journal of Drug Development and Research 2013; 5(1):297–304 (www.ijddr.in/Dacuments/8/38.pdf).
29. Haq F, Ullah R. Comparative determination of trace elements from *Allium sativum*, *Rheum australe* and *Terminalia chebula* by atomic absorption spectroscopy. International Journal of Biosciences 2011; 1(5):77–82 (innspub.net/wp-content/uploads/2012/01/IJB-V1-No-5-p77-82.pdf).
30. Öztürk M, Aydoğmuş-Öztürk F, Duru ME, Topçu G. Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant. Food Chemistry 2007; 103:623–630.
31. Singh PP, Ambika, Chauhan SMS. Activity-guided isolation of antioxidants from the roots of *Rheum emodi*. Natural Products Research 2013; 27(10):946–949.
32. Rajkumar V, Guha G, Kumar RA. Antioxidant and anti-cancer potentials of *Rheum emodi* rhizome extracts. Evidence-Based Complementary and Alternative Medicine, 2011. (http://www.hindawi.com/journals/ecam/2011/697986/).
33. Jahan N, Khalil-ur-Rahman, Ali S, Asi MR. Phenolic acid and flavonol contents of gemmo-modified and native extracts of some indigenous medicinal plants. Pakistan Journal of Botany 2013; 45(5):1515–1519. (www.pakbs.org/pjbot/PDFs/45(5)/05.pdf).