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Biologically Active Substances in Buckwheat (*Fagopyrum tataricum* L.) Cultivated in Mongolia

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Abstract

From thoroughly air-dried samples of buckwheat plant, we revealed a biochemical composition of 14 components. By thin layer chromatography and quantitative analysis methods we showed that buckwheat has 7 kinds of alkaloids with one dominating alkaloid, and the total weight of all alkaloids equals 0.05%. We also determined the aboveground parts of buckwheat contain the following substances: rutin-3.14%, fat-0.91%, protein-8.23%, carbohydrate-18.52%, monosaccharide-0.37%, disaccharides-1.11%, vitamin C-0.02%, ash-10.57%, acidity-0.05, carotine-15.6mg, cellulose-40.8%, tannin-1.70%, soluble pectins-0.266%, insoluble pectins-0.507%, total amount of alkaloids-0.05%.

Key words: alkaloid, *Fagopyrum tataricum*, flavonoids, Mongolia, pectin, rutin, vitamin

Introduction

Buckwheat (*Fagopyrum tataricum* L.) is a common weed plant in Mongolia. It has been widely used in both Western and Eastern medicines for a long time. Water and spirit extracts of buckwheat have been used for treatment of diseases affecting bronchial and tracheal organs, typhus, vitamin P deficiency, and mechanical developmental problems. This plant contains iron, calcium, phosphorus, citric acid, P, B1 and B2 vitamins and rutin (Tsitsin & Anichkov, 1962). Butin is a flavone compound that is active in treatments for increased permeability and brittleness of capillaries; it can accelerate cell proliferations, and prevent the agglutination of blood cells. It can also enhance vitamin C accumulation and reduce blood fat and cholesterols in humans. Also, it may be very useful for the prevention and treatment of hypertension, arteriosclerosis and diabetes (Wang *et al*., 1995). Buckwheat seeds can be used for human food and livestock feed, especially in pasturelands where bees help disperse the seeds (Tserenbaljid, 2002).

Buckwheat belongs to the family Polygonaceae and is a cross-pollinated plant, and insects are the major pollinators. Buckwheat can be grown under different climatic conditions on a wide variety of soil types, and can be planted at almost any time during the growing season (Taylor, 2004). Buckwheat is an annual herb, 15-70 cm in height, with erect stems branched, with longitudinal striations and 3-8 cm long triangular shaped leaves.

Most of higher plants contain a characteristic pattern of flavone and flavonol glycosides in their leaf or flower, and these substances are ideal taxonomic markers which can be used for plant taxonomy, hybridization or phytogeography (Wang *et al*., 1995). The term 'phenolic compound' embraces a wide range of plant substances which possess an aromatic ring bearing one or more hydroxyl constituents in common. Phenolic substances tend to be water-soluble, since they frequently occur combined with sugar as glycosides, and are usually located in the cell vacuole. The majority of phenolic compounds (especially flavonoids) can be detected on chromatograms by their fluorescence in UV light, the colors being intensified or changed by fuming the papers with ammonia vapor. Because phenolic pigments are visibly colored they are particularly easily monitored during their isolation and purification (Wang *et al*., 1995). Phenolic compounds are all aromatic, so they show intense absorption in the UV region of the spectrum (Harborne, 1976).

Flavonoids are structurally derived from the parent flavone substance, and are mainly water soluble compounds. They can be extracted with

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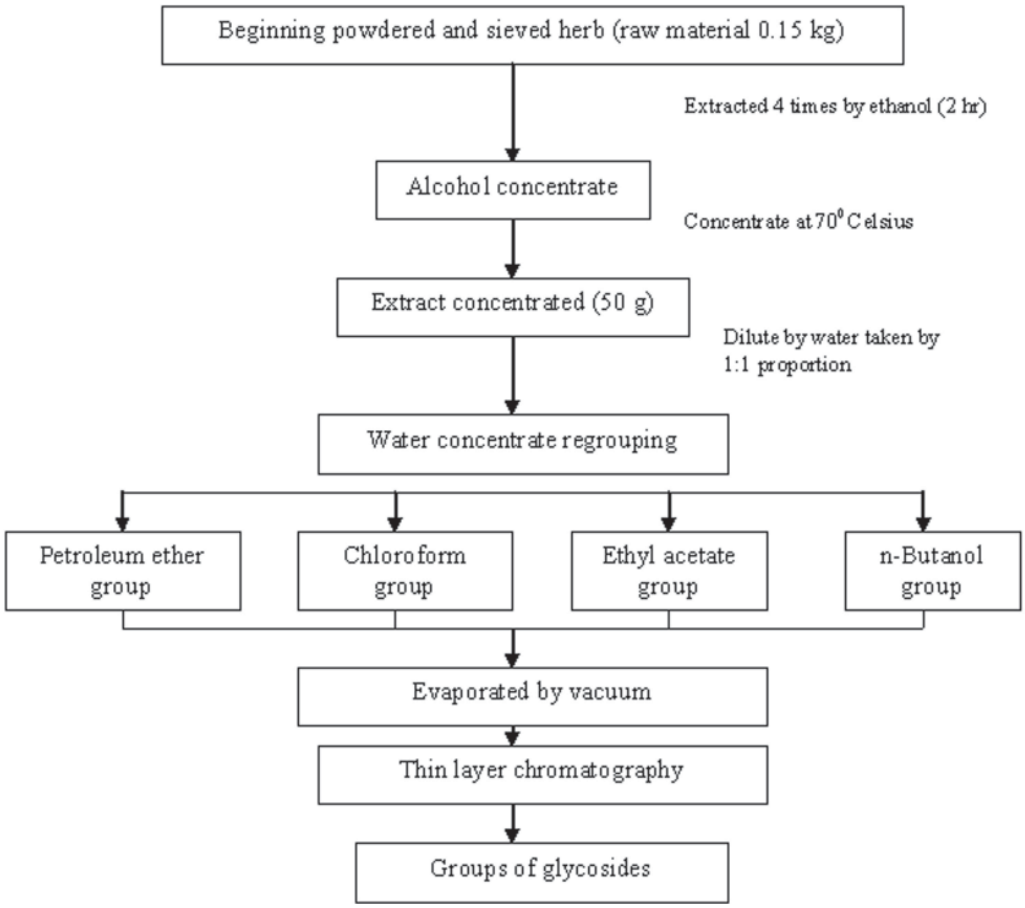
70% ethanol and remain in the aqueous layer, following extract with petroleum ether. Flavonoids are generally present in plants bound to sugar as glycosides and any flavonoid aglycone may occur in a single plant in several glycosidic combinations. For this reason, when analyzing flavonoids, it is usually better to examine the aglycones present in hydrolyzed plant extracts before considering the complexity of glycosides that may be present in the original extract. Flavonoids exist in all vascular plants, usually as mixtures; it is very rare to find only a single flavonoid component in a plant tissue (Harborne, 1976; Obendorf *et al*., 1993). Flavonol and quercetin are widely distributed in buckwheat. These substances have a positive influence on the ability of humans to resist malignant tumors (Maksutina, 1985), hence a point of interest for this research. So, the purpose of this research was to derive pure natural and biologically active compounds from buckwheat, and to investigate proper ways for their use as food additives and medicinal substances for treatments of humans.

Materials and Methods

The samples of buckwheat used for this research were collected from the field of "Bayandulaan Uul" Co. Ltd in Tsagaan Nuur district, Selenge province, in its blooming period at the end of July 2002. The above- and belowground parts of those samples were separated, cleaned, and dried in the open air. Protein was determined using the Ganning method; b-carotenoid content was determined by applying low-temperature vacuum evaporation, extraction with diethyl-ether, and absorption on fixed aluminum layered hexane. Total amount of carotenoids in the samples was estimated by applying a spectrophotometric method in the range of visible light absorption in the 418–420 nm regions. Vitamin C was determined by titration with 2.6-dichlorophenolindolephenol. Oil content was determined by the Soxhlet method (Homutov & Groyn, 1987). Sacchharides were determined by the method of Bertrane, and saccharides, soluble pectin, and protopectin were saponified. Pectin was calculated by weighing

Figure 1. Steps of method for separating biologically active compounds from buckwheat



sediments of pectate calcium taken to a constant weight. Total amount of alkaloids were determined by thin layer chromotography (Harborne, 1976).

To analyze flavonoids, 0.15 kg of dry buckwheat sample was extracted 4 or 5 times with 70% ethanol solution. After filtering, the extract was distilled at a low temperature vacuum with low pressure to produce a condensed extract. It was then diluted with distilled water. To separate high molecular compounds and to increase the resolution, four organic solutions were used as treatments (chloroform: methanol; 9:1, 8:2). Each substance was extracted individually after checking by thin layer chromatography with butanol: acetic acid: water (4:1:2) buffer system. Each fraction was checked by thin layer chromatography and after spraying with a 2% vanillin, 10% H_2SO_4 indicator solvent, was heated up to 120°C. Dominating pink spots appearing at this stage show the existence of rutin and its glycoside.

To separate rutin and quercetin extracted from buckwheat, we applied these column and thin layer chromatography methods. The extracted rutins and quercetins were identified by ultraviolet absorption and infrared spectrum and nuclear magnetic resonance spectroscopy.

To isolate biologically active compounds from buckwheat we used 0.15 kg of air dried sample. After grinding the sample into a powder, weighing, and processing in water for 2.5 hr in 90-100°C, the water solution was condensed in a rotator vacuum. After dissolving the condensed products in a 96% alcohol solution, rutin was isolated as shown in figure 1.

The steps for rutin and quercetin isolation are shown in figure 2.

Results

From thoroughly air-dried samples of buckwheat, we revealed the 14 biochemical components. By thin layer chromatography and quantitative analysis methods we determined that buckwheat has 7 kinds of alkaloids with one dominating alkaloid, and the total weight of all alkaloids was equal to 0.05%. Buckwheat rutin equals 3.014% and a procedure is presented for processing and isolating this substance in pure state. As a result of this experimental work we demonstrated the potential production of pure natural food additives.

The biochemical components determined from the above ground parts of buckwheat are shown in Table 1.

Quercetin glycoside and rutin were found in the ethyl acetate group. Therefore we used this group for further research. The steps for rutin and quercetin isolation are shown in figure 2.

It is visible that from 0.15 kg of dry buckwheat we derived 1.16 g of pure rutin and 0.03 g of pure quercetin. It could, therefore, be possible to produce about 7.73 g of pure rutin and 0.2 g of pure quercetin from 1 kg of buckwheat.

Table 1. Biochemical components identified from buckwheat.

Biochemical components		Content
Rutin		3.14 %
Fat		0.91 %
Carbohydrate		18.52 %
Monosaccharide		0.37 %
Disaccharides		1.11 %
Protein		8.23 %
Vitamin C		0.02 %
Ash		10.57 %
Acidity		0.05 %
Carotene		15.6 mg
Cellulose		40.8 mg
Alkaloid		0.05%
Tannin		1.70 %
Soluble-pectin		0.266 %
Insoluble-pectin	With HCl	0.507 %
	With citric ammon acid	0.351 %

Despite buckwheat being widely used in the treatment of many illnesses, there is no detailed phytochemical analysis of this plant species. Furthermore, we know that this plant can be cultivated to increase plant material for large scale isolations of its biologically active compounds, and consequently applied to medical treatments such as blood vessel related diseases, hemorrhage, or to improve the active impact of hormones, including adrenaline.

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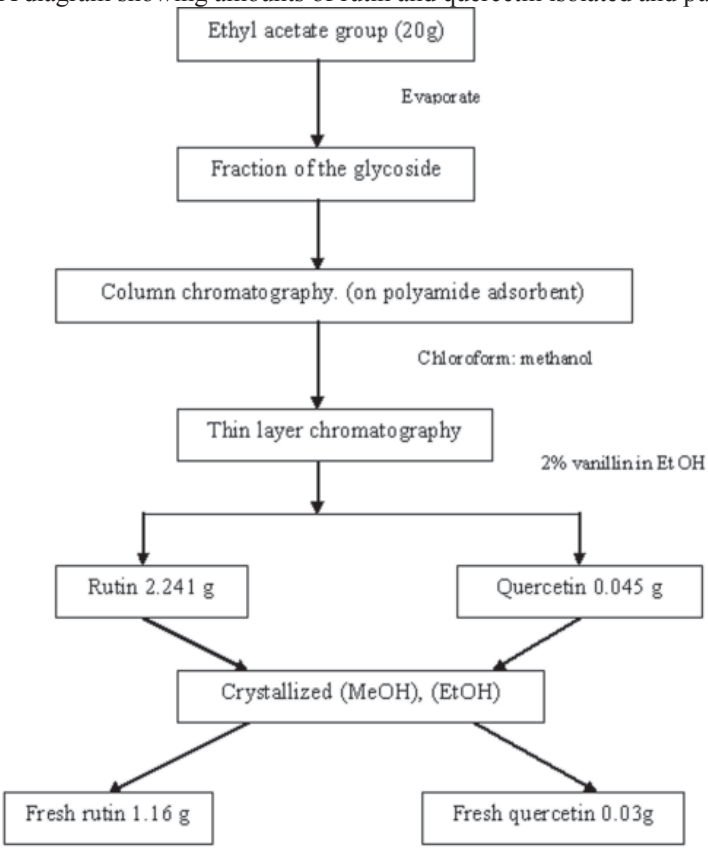
Figure. 2. A diagram showing amounts of rutin and quercetin isolated and purified from



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Хураангуй

Татаар сагаг (*Fagopyrum tataricum*)-ийн дээжинд биохимийн анализ хийсний дүнд 14 төрлийн нэгдлийг илрүүллээ. Нимгэн үет хромотографийн шинжилгээ болон тооны анализын судалгаагаар татаар сагагт нэг төрөл нь зонхилсон 7 төрлийн алкалойдыг илрүүлсэн бөгөөд тэдгээрийн нийт жингийн хэмжээ 0.05% болохыг илрүүлэв. Түүнчлэн татаар сагагийн газрын дээрх эрхтнүүдэд рутин 3.14%, тослог 0.91%, уураг 8.23%, карбогидрат 18.52%, моносахарид 0.37%, дисахарид 1.11%, витамин С 0.02%, үнс 10.57%, хүчиллэг 0.05, каротин 15.6 тг, целлюлоз 40.8%, таннин 1.70%, уусдаг пектин 0.266%, уусдаггүй пектин 0.507%, нийт алкалойд 0.05% агуулагддаг болохыг тодорхойлов.

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