

## Application of *Swertia Chirayita* (Roxb. ex Fleming) Karst in Wheat Flour to Design Health Food

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*Swertia Chirayita* often known as *Chiraito* is a highly traded medicinal plant of Nepal. Its use in food industries is still under research. Application of the medicinal herb in bread prepared from wheat flour (550 types, 431  $\mu\text{m}$  particle sized with moisture 12.5%) was assessed to evaluate sensory and physiochemical qualities. The antioxidant activity of the *Chiraito* was found to be 62% at 500  $\mu\text{g/ml}$  and 15% at 100  $\mu\text{g/ml}$  by radical scavenging method which shows *Chiraito* as the most significant antioxidants potential. Likewise, antibacterial activity of the herb extract also showed significantly inhibitory to selected pathogenic microorganism. Finding its functional properties, *Chiraito* powder (669  $\mu\text{m}$  particle sized) with 1.0 %, 0.10%, 0.00% and 0.01% respectively were added in wheat flour to make four different type of bread and carried out sensory evaluation using 9-points (Hedonic Rating Test). The data were statistically analyzed using SPSS for Analysis of Variance (ANOVA) at 5% level of significance. The overall quality of the finished products also showed that *Chiraito*, as herb extract didn't affect physico-chemical properties of the product which was measured in terms of dough yield, bread volume, elasticity, acid degree, water content, crumb porosity, texture, smell and taste. Overall acceptability showed that bread (Sample D) with 0.010% was superior and liked the most. Since no entrance of *Chiraito* herb in modern food industries yet, the research would support production and commercialization of innovative functional health food globally.

**Keywords:** Antioxidant, Bread, *Chiraito*, Inhibition, Wheat flour

### Introduction

Bread is a nutritional, culinary, social and environmental mess and one of the mankind's important discoveries, possibly ranking alongside the discovery of fire and the invention of the wheel. Bread has played a key role in the development of mankind and is a principle source of nutrition. Historically, it has contributed to language social structuring and spiritual imagery (Cauvain and Young, 2006). Recently Nepal earthquake has affected Nepalese livelihood and thousands of people have been killed and injured. It was the worst natural disaster to strike Nepal. In this situation, Agriculture being one of the main sectors of Nepal supposed to play significant role to support and build new Nepal. Sustainable utilization of Agro byproducts and Medicinal herb plants has been thought to minimize nutritional problems for economic development. In this context, medicinal native plants such as bitter herbs, *Chiraito* as selective herbal plant, can play vital role as

functional food for healing practices. It ranks high in terms of medicinal importance and drug value among the local Nepalese folklore disorders (Phoboo, S. 2012). Its usefulness is also being utilized by *Ayurveda* and *Yunani* medicinal practices. Its use can be a possible attempt to minimize the existing illegal trade from himalayan region. Minimization of its loss is being sought hereby inventing and developing innovative functional health food products incorporating in cereal flour. Oral communication among local people mentioned that the herb has antioxidant and antimicrobial properties which is hereby researched and healthy functional food is designed by incorporating in wheat flour for therapeutic use. So it definitely may convey positive impact to entrepreneurs and consumers at their lifestyle (food habit) because of its health benefit bioactive component presence. Awareness and Scale up of market through Cooperative partnership with academicians, processors, health workers and consumers activists may play vital role for sustainability.

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## Materials and Methods

### Materials

Refined wheat flour (*maida*) used for bread preparation was obtained from Kathmandu Maida Mills (KMM) Balaju, Kathmandu, whereas *Chiraito* was collected from Rasuwa district at the Central zone of Nepal. The plant was taken to National Herbarium Laboratory under the Department of Plant Resource (DoPR), Godawari, Lalitpur for plant identification. The senior botanist Mr. Binod Basnet, Chief of laboratory, identified it as *Swertia Chirayita* (Roxb. ex Fleming) karst. The fresh plant was taken and then dried, pressed and grinded to make powder. Other ingredients like sugar, salt, shortening (Medalia), Gluten (Vital), yeast (Victoria Instant Dry Baker's Yeast), improver (Tower), Calcium propionate, Lecithin as emulsifier (Stampfli, L and Nersten, B. 2000) and water were calculated and blended at mixture (EBPM-20L, Guangzhou South star Machine Facilities Co. Ltd. China) to make smooth dough. The dough was fermented and baked in oven (Digital Rotary Rack Oven "Ebro 68" (Guangzhou South star Machine Facilities Co. Ltd. China) at 180°C for 35 minutes followed by quality assessment. The samples were packed after cooling at room temperature in food grade polyethylene bags for quality assessment at Central Food Research Laboratory (CFRL), Kathmandu.

### Methods

#### Phyto-Chemical analysis of *Chiraito*

Phyto-chemicals screening of the sample was carried out according to the methods of (Asthana, RK; Sharma NK. And Kulshrestha, DK; 1991). The different plant parts (Root, Leaves and Stem) were mixed with 1:1:1 ratio and its extracts obtained from Cold methanol (laboratory grade) were screened for Alkaloids, Sterols, Terpenes, Tannins, Coumarin, Saponin, Glycosides and Quinones respectively.

#### Antimicrobial screening

The antimicrobial activity of the extract was determined by Kirby-Bauer method in agar well diffusion (Kirtikar KR and Basu B.D. 1996). The stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h in an incubator. Then agar plates of the Muller Hilton's Agar media were prepared. Each plate was inoculated by swabbing with bacterial suspension or with 18 h fresh culture (10<sup>5</sup>–10<sup>6</sup> colony forming unit "CFU"/ml), which was swabbed evenly on the surface of solid agar media by the help of cotton swab. All the plates were incubated at 37°C for 24 h and then during observation after 24 h, the diameter of the zone of inhibition was measured in millimeter. All the equipment used like media, Petri plates, cotton swab etc.,

were sterilized before performing the experiments. As well as proper labeling of the petri plates were done before performing. All the determination of antibacterial activities of plant extracts were also done in triplicates. After 20 min, wells of 6 mm diameter were made in solid agar medium with the help of gel puncher and filled with 50 µl of various test samples of *Chiraito*. The positive control wells were filled with *Chloramphenicol* as Standard drug.

1. *Escherichia coli*
2. *Salmonella typhi*
3. *Citrobacter freundii*
4. *Klebsiella pneumonia*
5. *Staphylococcus aureus*

The test microorganisms as shown above were obtained and screened at the laboratory of the Department of Biotechnology Kathmandu University (Dhulikhel).

#### Antioxidant activity

Antioxidant activity was performed in Nist College, Khusibu / Kathmandu. The rate of reduction of a chemical reaction upon addition of Free radical scavenging assay using 1,1-diphenyl-2-picrylhydrazyl radical ((DPPH) Sigma-Aldrich) was used as an indicator of antioxidant properties at 517 nm. 25 mg of dry crude extracts were taken and dissolved in distilled methanol and diluted up to 50 ml. was carried out according to Von Gadov *et al.* 1997. From the stock solution 100 and 500 µg/ml was prepared. Ascorbic acid was used as a standard. 5 ml of each solution was taken in a test tube and 1 ml of 0.001 M of DPPH solution was added to it. All these solutions were kept in dark for 30 min. Also 5 ml methanol was taken and 1 ml of DPPH solution was added, for control (A<sub>0</sub>) solution. At the end of incubation period the mixtures were examined for the antioxidant activity using Optima UV Visible spectrophotometer at wavelength of 517 nm. The experiments were performed with triplicate readings. Percentage DPPH inhibition was determined using

$$\text{Antioxidant activity (\%)} = \frac{A_0}{A_1} \times 100$$

Where, A<sub>0</sub> is the absorbance of Control and A<sub>1</sub> is the absorbance of the sample

The physico-chemical properties of flour have been shown in the Table 1.

**Table 1. Physico-chemical properties of flour**

Parameters	Wheat flour
Moisture (%)	10.50
Ash (%)	0.554
Sedimentation (%)	10.00
Falling number (%)	349.50
Gluten index (%)	98.35
Farinograph (500 FU)	60.50

The moisture was determined using hot air oven, the world standard method (AACC, 2000). Similarly, ash, to determine inorganic substances, was also determined using ICC standard based on 104/1. Sedimentation value that provides information on the protein quantity and quality of flour samples, its value can be in the range of 20 or less for low protein flour with weak gluten to as high as 70 or more for high protein with strong gluten (Paul, 2000). Sedimentation value of wheat and composite flour of wheat and millet were measured respectively using lactic acid solution during a standard time interval based on AACC, ICC No 116/1. The falling number was measured using the world standard alpha amylase activity test- AACC No. 56-81B, ICC No. 107/1, ISO/DIS 3093, ASBC. Gluten is the functional component of protein and determines processing characteristics of flour. It was measured using the ICC standard No 155 and 158, AACC method No 38-12, ISO 7495. Force or torque during mixing a small quantity of dough that characterizes quality and mixing behavior of tested flour was determined by Farinograph Units (FU) following ICC standard No. 115/1. It was important to measure percentage of water absorption, dough development time (DDT), dough stability (DS) and mixing tolerance index (TI). The product formulation and process control are hereby shown in the Table 2.0 and 3.0 respectively.

**Table 2. Product formulation**

Ingredients	Type of products			
	A	B	Control	D
Wheat (flour %)	100	100	100	100
Chiraito (%)	1.0	0.1	0.0	0.01
Water (%)	60.5	60.5	60.5	60.5
Salt (%)	1.67	1.67	1.67	1.67
Yeast	1.33	1.33	1.33	1.33
Sugar (%)	1	1	1	1
Lecithin(%)	0.65	0.65	0.65	0.65

The lab work of final product included measurement of overall physico-chemical properties such as water content, acid degree, compressibility, resilience and elasticity where as technological properties included dough yield, bread yield, bread volume yield and baking loss as shown in Table 4.0 The acid degree which is important to determine the physical state of gluten was measured by AACC (2000) method. Compressibility was measured in terms of texture using Penetrometer. The force was measured for 5 seconds and 115 seconds respectively which were monitored as read by scale on the penetrometer. The difference in force ( $\Delta K_m$ ) in 115 and 5 seconds indicated the force applied to the bread crumb ( $\Delta K_m = K_{m115} - K_{m5}$ ). This test was measured under the AACC, (2000) method.

**Table 3. Process control table**

Preparations	kneading time (Min)	4
	Temp after kneading (°C)	35
Primary fermentation	Time (Min)	15
	Temp (°C)	35
Secondary fermentations	Time (Min)	50
	Temp(°C)	35
	Step 1 –Temp (°C)	210
	Time (Min)	10
Baking	Step 2 –Temp (°C)	200
	Time (Min)	10
	Step 3 –Temp (°C)	180
	Time (Min)	15

### Sensory analysis

The results of sensory analysis were subjected for two-way ANOVA and LSD tests at 5% level of significance among formulations were made.

The data were statistically analyzed using SPSS for Analysis of Variance (ANOVA) at 5% level of significance. The overall quality of the finished products also showed that *Chiraito*, as herb extract didn't affect physico-chemical properties of the product which was measured in terms of dough yield, bread volume, elasticity, acid degree, water content, crumb porosity, texture, smell and taste. Overall acceptability showed that bread (Sample D) with 0.010% was superior.

### Results and Discussions

The *Chiraito* sample was collected from Rasuwa district of Nepal. The Phytochemical, antimicrobial, antioxidant properties of the herb plant was analyzed. Plant extract showed the presence of phytochemicals such as basic alkaloids, coumarin, glycosides, steroids, quinones, flavonoid and terpenoids. The antibacterial activity of the

extract showed significantly bioactive by inhibiting the growth of selected pathogenic microbial species for the test. The zone of inhibition (ZOI) shown by the extracts was comparable to the standard antibiotics. The antioxidant activity of the sample was found to be 62% at 500 µg/ml and 15% at 100 µg/ml by radical scavenging method and showed most significant antioxidants potential. Its incorporation in wheat flour to make bread and its sensory characteristic was also studied. The bread prepared using *Chiraito* at the rate of 0.01% was liked the most. Since *Chiraito* is one of the most useful medicinal herbs found in Nepal, its use in brewery is under research as hops substitute. Mangiferin, Swetiamerin and Amarogentin found in *Chiraito* is potential as functional supplement (Subedi, I and Kartki, T. 2018). Magniferin is named after *Mangifera indica*, the leaves of mango, which is reported to possess considerable hypoglycemic property due to this phytochemical (Muruganandan, S. 2005). Mangiferin has also been reported to show suppressive effects on blood lipids in diabetes (Leiro, J.M., 2003). It is reported as anti-proliferative, immunodilatory, cardiotoxic and diuretic properties (Andrea, A.C, 2005). Mangiferin significantly increased heart tissue phospholipids in isoproterenol induced cardio-toxic rats suggesting cardioprotective and hypolipidemic effects (Nair and Devi, 2006). The infusion of *Swertia chirayita* is locally used in Nepal as an anti-diabetic (Oral local communication). A review of literature indicates that mangiferin seem to have several modes of actions for counteracting diabetes and its complications; directly by stimulating insulin production, enhancing glycolytic enzymes, inhibiting glucosidase and other

enzymes such as maltase, sucrase, isomaltase and aldose reductase and indirectly by its antioxidant capacity, analgesic, anti-inflammatory and antiatherogenic (Muruganandan,S., 2005), cardioprotective and antihyperlipidemic properties (Nair and Devi 2006). Swertiamarin, has been reported to possess a number of pharmacological properties such as hepatoprotective and antidiabetic/anti-inflammatory, free radical scavenging and antispastic (Vaijanathappa and Badami, 2009). Swertiamarin has also been reported as a potent lipid lowering agent comparable to the clinical drug atorvastatin which may also contribute to its cardio-protective and anti-atherosclerotic role (Vaidya, H. 2009). Swertiamarin has been reported to have very low toxicity and is anti-bacterial (Sultana and Amhed, 2013). The result from this study indicates that swertiamarin and its derivatives are present in all plant parts of *Chiraito* and supposed to possess functional to health benefit. Amarogentin, the bitterest compound known to mankind, is known as poimerase inhibitor (Ray, S. 1996), chemopreventive and is reported to have anti-leishmanial and gastroprotective properties (Medda,S. 1999).

The overall quality of the finished products also showed that *Chiraito*, as herb extract doesn't affect its physico-chemical properties, which has been tabulated in Table 4.

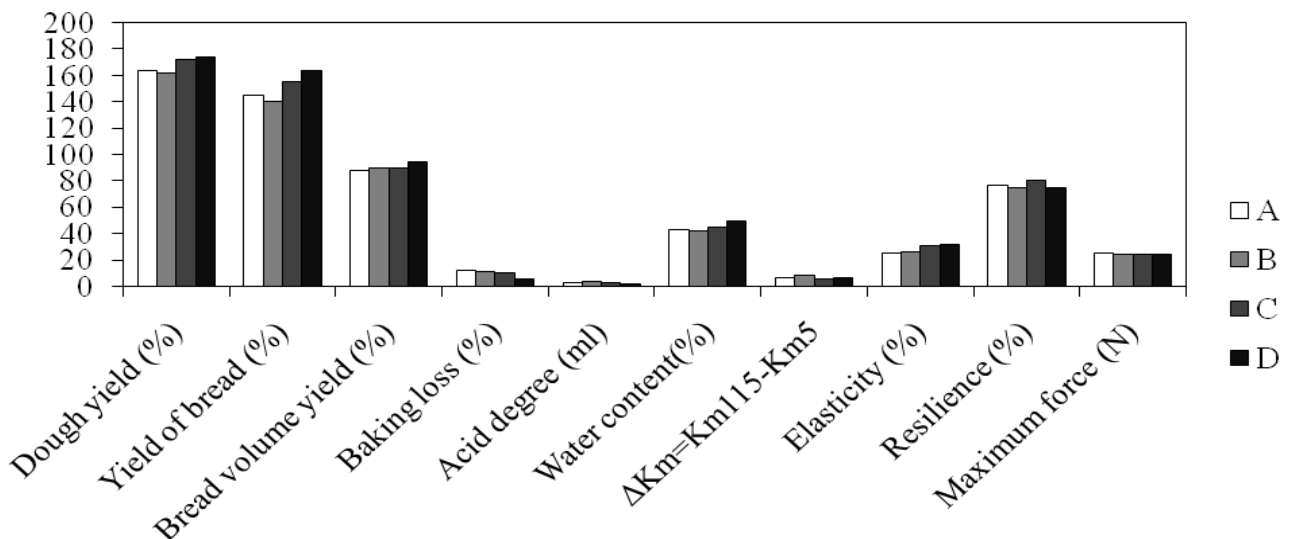


Figure 1. Overall quality of the finished product

**Table 4. Overall physico-chemical properties of the products**

Parameters	Samples			
	A	B	Control	D
Dough yield (%)	164	162	172	174
Yield of bread (%)	145	140	155	164
Bread volume yield (%)	88.1	89.5	90	94.1
Baking loss (%)	12	11	10	6
Acid degree(ml)	2.8	3.3	2.6	2.1
Water content(%)	42.6	42.1	44.7	49.9
$\Delta K_m = K_m 15 - K_m$	7	8	6	7
Elasticity (%)	25.5	26.5	31	32
Resilience (%)	76.4	75	80.7	75
Maximum force (N)	25.56	24.05	24.50	24.4

### Conclusions

In order to develop the baking science in flour confection technology, application of *Swertia chiraita* from 0 to 1.0 % in fine wheat flour (431  $\mu\text{m}$ ) for the production of bread has been investigated. The *Chiraito* herb was tested to assess its antimicrobial and antioxidant property prior to application in flour confection. Then it was determined to add in wheat flour and bread was prepared. The products so prepared were subjected to sensory evaluation using 9-points (Hedonic Rating Test) and data were statistically analyzed using SPSS for Analysis of Variance (ANOVA) at 5% level of significance. The Statistical sensory analysis (Color, Crumb, Texture, Taste, Crust and Overall acceptability) showed that bread (Sample D) product supplemented with 0.010% *Chiraito* powder was found to be significantly superior among all bread formulations. So it can be recommended that wheat flour has a good potential for use as a functional agent in bakery products to design health foods.

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