JNCS Journal of Nepal Chemical Society
ISSN: 2091-0304 (print)
DOI: 10.3126/jncs.v44i1.62686
February 2024, Vol. 44, No. 1, 122-133
RESEARCH ARTICLE

The antioxidant property of *Urtica dioica* L leaf and Formulation development of its Tablet

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Submitted: 06/28/2023, revised: 10/15/2023, accepted: 10/22/202

Abstract

Stinging neetle (*Urtica dioica* L.) is a medicinal plant belonging to Urticaceae which is locally used for rheumatism, and skin ailments, diuretic, antidiabetic, and also as a food in Nepal. This study explores the antioxidant properties of the plant and formulation assessment of herbal tablets from *Urtica dioica* L. ethanolic extracts. The Folin-Ciocalteu method determined the total phenolic content. The DPPH assay demonstrated an antioxidant property with IC_{50} of 37.57 µg/mL, compared to the standard ascorbic acid with an IC_{50} value of 21.23 µg/ml. The total phenolic content of the extract was found to be 115.71 mg/g of the Gallic acid equivalent. Herbal tablets were formulated using the direct compression method. The tablets demonstrated good flow properties, uniformity, appropriate hardness, and low friability. The findings highlight the potential of *Urtica dioica* L extract as bio-prospecting for effective herbal antioxidants tablets.

Keywords: *Utrica Dioca L*, Antioxidant, Soxhlet apparatus, DPPH, FC, Tablet

Introduction

Nepalese medicinal plants can be utilized for various medicinal purposes such as antioxidant, analgesic, antimicrobial, and antidiabetic due to their unique chemical substances with different compositions [1-4]. *Urtica dioica* L belongs to *the* Urticaceae family, and possesses acetylcholine, formic acid, histamine, and serotonin, causing an itchy, burning rash in humans and other animals that may last up to 12 hours. The leaves also contains abundant amounts of natural phenolic compounds, such as flavonoids, phenolic acids, anthocyanins, and other phenols, which may be hypotensive, anti-inflammatory, to be useful in the therapy of prostatic hyperplasia, diuretic, immunomodulatory, to alleviate rheumatic pain, and to serve as an adjuvant therapeutic agent in rheumatoid arthritis [5,6]. This plant has been uses as a wild leafy vegetable for centuries in Nepal for its good calorific value which boost growth and enhance the immunity. Plethora of studies shows that plant maturation leads to changes in their

chemical composition [7]. Researchers report that rutin is the predominant phenolic compound in stinging nettle leaves possessing a potent anti-inflammatory property [8]. Terpene diols, terpene diol glucosides, α-tocopherol, and five monoterpenoid components have also been detected in nettle leaves [9]. However, it was not fully studied on chemical composition on Nepalese originated sample. The aim of this research is to evaluate the antioxidant property in best suitable solvent, and then prepare a tablet from its extract.

Material and methods

Plant material

A sample of *Urtica dioica* L. was collected, washed, and separated into roots, stalks, and leaves. Then leaves were kept in a zip lock bag at −20°C for inhibition of air contact before analysis [16]. To increase extraction yield, dried nettle leaves were ground to powder using a mortar and pestle and sieved through a 0.355 mm riddle to produce plant material of uniform particle size.

Calculation of yield percentage on various solvents

Solvents ethanol, methanol, and ethyl acetate were used to optimize the high yield and respectively best antioxidant property. Total 1 gm of sample was taken and 10 ml of solvent was added in different beakers containing the sample. Solvent was evaporated in a water bath and the weight of extracts was taken and then percentage yield was calculated. As an indicator for enriched amount of phytochemicals, tannin content was considered which was observed by 5% FeCl3 test during the screening.

Optimization of extraction solvent

After extraction with different solvents, based on rich amount of total phenolics, ethanol was selected for further extraction process. Total 50 gm of dried leaf was weighed and poured with 200 mL of ethanol in a flask which was extracted by Soxhlet apparatus for 4 hours. The extract was filtered and evaporated in the rotavapor (Buchi company).

Ethanolic Extraction Procedure

After trials of different types of extraction methods, 100% ethanol was used as an extraction liquid. 50 grams of dried nettle was weighed and poured with 200 mL of ethanol in a flask which was extracted by Soxhlet apparatus for 4 hours. The process is repeated 3-4 times for better results. After four hours of extraction the extracts are filtered through filter paper and then stored in a dark and cool place before analysis. Phenolic extracts can be prepared by soaking dried powder plants with 100% ethanol for 4-5 days. Then after 5 days, the supernatant liquid was filtered and evaporated in the rotavapor at 40 to 45 degrees Celsius. The information about the sample profile is given below in Figures 1, 2, and 3.

Fig 1. *Urtica Dioica Plant and Sample under Dry*

Fig *2.* Sample Extraction and Evaporation

Fig 3. Phytochemical analysis of *Urtica Dioica* L leaves

Determination of antioxidant potential

DPPH radical scavenging activity was assessed for the determination of the antioxidant power of *Urtica dioica* L [10]. A DPPH solution of 100µmol was prepared by dissolving 1.94 mg of DPPH in 100 ml of ethanol and wrapped with aluminum foil which was freshly prepared. 500 ppm stock solution of ascorbic acid/plant extract was prepared by dissolving 50 mg ascorbic acid/plant extract in 100 ml ethanol. Standard/test solutions 10, 15, 20, 25, and 30 ppm of ascorbic acid/plant extract were prepared from stock solution by dilution for Standard and sample solutions respectively. The test sample was prepared by adding 5 ml ethanolic plant extract solution and 5 ml of 100µmol ethanolic DPPH solution respectively from each concentration. Control was prepared by taking 5ml ascorbic acid and5ml of 100µmol of ethanolic DPPH solution. Ethanol as blank. These samples were well shaken and kept in the dark for 30 min at room temperature. immediately covered with aluminum foil. The absorbance was measured at 517 nm using a UV spectrophotometer after 30 minutes. IC_{50} was calculated by % inhibition. The capability to scavenge DPPH radical was expressed as a radical scavenging percentage using the following equation:

Percentage Scavenging $=\frac{Ac-As}{Ac}*100$, Where Ac = Absorbance of control, As = Absorbance of sample solution

Estimation of Total Phenolic Contents:

Total phenolic content analysis of nettle extracts was done by using the Folin-Ciocalteu (FC) method as per previous method with slight modification [10,11]. FC phenol reagent was prepared by dissolving 10 g of sodium tungsten and 2.5 g of sodium molybdate in 70 ml of water. FC reagent prepared was diluted with 10% distilled water for use. Add 5 ml of 85% phosphoric acid and 10 ml of concentrated hydrochloric acid. Reflux for 10 hours. Add 15 g of lithium sulfate, 5 ml of water, and 1 drop of bromine. Reflux for 15 minutes. Cool to room temperature and bring to 100 ml with water. 500 ppm stock solution of Gallic acid/ plant extract was prepared by dissolving 50 mg ascorbic acid/plant extract in 100 ml ethanol. Standard solution/Test solutions 10, 15, 20, 25, and 30 ppm of Gallic acid/plant extract

were prepared from stock solution by dilution for Standard and sample solution respectively. The test sample was prepared by adding 2.5ml FC reagent into 0.5ml nettle extract stock solution. At the end of this period, 2.5ml of 7.5% Na_2CO_3 solution was added respectively from each concentration. In this way, phenolic hydroxyl groups could give H to water. Control was prepared by adding 2.5ml FC reagent into 0.5ml gallic acid stock solution. At the end of this period, 2.5ml of 7.5% Na2CO3 solution was added respectively from each concentration. Blank as 0.5 ml ethanol, 2.5 ml 5% Folin-Ciocalteu reagent dissolved in water, and 2.5 ml of 7.5% of sodium carbonate. All this mixture was incubated for 45 min in an incubator for 45 degrees Celsius. After incubation, the development of blue color was observed. Samples and standard absorbance were measured at 765 nm using UV/ Visible Spectrophotometer against blank. Total phenolic content was expressed percentage (w/w) and calculated using the formula:

C = c V/m**,** Where, C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in ml, m = mass of extract in gram. The linear regression equation for a straight line is, Y = mx + c where, Y = absorbance of extract, m = slope of the calibration curve, x = concentration of extract, c = intercept. Using this regression equation, concentrations of extracts were calculated. From the calculated values of concentration of each extract, the total phenolic content was calculated.

Formulation of *Urtica Dioica* L Tablet

Formulation of tablets from the phenolic extract was conducted by direct compression method. Direct compression refers to the practice of compressing tablets directly from powdered active drug components and appropriate excipients into a solid compact without using the granulation process. These involve mixing and processing formulation ingredients and then compressing them into tablets. To achieve the perfect formulation, we performed the "Hit and Trial" method, experimenting with different formulations to find the best one. We crafted five unique formulations through this approach, tweaking the components and proportions each time.

Table 1. 'Formulation A' for the tablet preparation from *Utrica dioica* L extract

SN.	Ingredient	Weight per tablet in mg	Weight for 250 tablets
i.	API	25	6250mg
ii.	Lactose (Filler)	220	55000 mg
iii.	(Adsorbent) Aerosol (5%)	30	7500 mg
iv.	(Lubricant) Talc (10%)	15	3750 mg
v.	(Disintegrate) SSG (2.5%)	7.5	1875 mg
vi.	(Binder) PVP K30 (2.5%)	7.5	1875 mg
	Total	**305 mg**	**76.25 gm**

Table 2: 'Formulation B' for the tablet preparation from *Utrica dioica* L extract

SN.	Ingredient	Weight per tablet in mg	Weight for 250 tablets
i.	API	18	4500mg
ii.	Lactose (Filler)	230	575000 mg
iii.	(Adsorbent) Aerosol (5%)	30	7500 mg
iv.	(Lubricant) Talc (10%)	20	5000 mg
v.	(Disintegrate) SSG (2.5%)	5	1250 mg
vi.	(Binder) PVP K30 (2.5%)	7.5	1875 mg
	Total	**310mg**	**77.625gm**

Table 3. 'Formulation C' for the tablet preparation from *Utrica dioica* L extract

SN.	Ingredient	Weight per tablet in mg	Weight for 250 tablets
i.	API	22	5500mg
ii.	Lactose (Filler)	200	50000 mg
iii.	(Adsorbent) Aerosol (5%)	35	8750 mg
iv.	(Lubricant) Talc (10%)	10	2500mg
v.	(Disintegrate) SSG (2.5%)	10	2500 mg
vi.	(Binder) PVP K30 (2.5%)	10	2500 mg
	Total	**287.5mg**	**71.75gm**

Table 4. 'Formulation ' for the tablet preparation from *Utrica dioica* L extract

SN.	Ingredient	Weight per tablet in mg	Weight for 250 tablets
i.	API	19	47500mg (4.75gm)
ii.	Lactose (Filler)	240	60000 mg (60g)
iii.	(Adsorbent) Aerosol (5%)	25	6250 mg (6.25gm)
iv.	(Lubricant) Talc (10%)	18	4500mg (4.5gm)
v.	(Disintegrate) SSG (2.5%)	8	2000 mg (2gm)
vi.	(Binder) PVP K30 (2.5%)	7	1750 mg (1.75gm)
	Total	**300 mg**	**75 gm**

Table 5. 'Formulation E' for the tablet preparation from *Utrica dioica* L extract

SN.	Ingredient	Weight per tablet in mg	Weight for 250 tablets
i.	API	20	5000 mg
ii.	Lactose (Filler)	220	55000 mg
iii.	(Adsorbent) Aerosol (5%)	30	7500 mg
iv.	(Lubricant) Talc (10%)	15	3750 mg
v.	(Disintegrate) SSG (2.5%)	7.5	1875 mg
vi.	(Binder) PVP K30 (2.5%)	7.5	1875 mg
	Total	**300 mg**	**75 gm**

After creating all five formulations, we established criteria such as effectiveness in achieving desired properties, stability, and compatibility with our intended application.

Through a thorough evaluation process, we carefully assessed each formulation based on these criteria and took into consideration formulation E as a master formula for the formulation of tablets.

The extract was weight in required amount and was mixed with other excipients in required amount. The ethanolic plant extract taken was mixed properly with required amount of lactose (filler) at first which was then followed by addition of talc and aerosol. Then these mixers were allowed to dry in an oven at a controlled temperature for about 10 min and were sieved using 0.355 µm mesh screen. The SSG was added to the mixture and was mixed homogenously in which PVP K 30 was added at last. The mixture was spread evenly on trays and dried in an oven at a controlled temperature not exceeding 55 ^{o}C to a consistent weight or constant moisture content and

was followed by sieving using a 0.355 µm mesh screen. The resulting mixture was kept in a poly bag mixed properly and was kept in desiccators for further use. Tablets were formed through direct compression by compressing the powder in compression machines. The pictures of herbal powder and tablets are shown in Figure 3.

Evaluation of Tablets

The powder was tested for its flow ability properties by calculating Hausner's Ratio and Carr's Index after measuring Bulk and Tapped densities using the formula. The granule size distribution test was done after adding the external phase. The prepared tablets were evaluated for their appearance, weight variation, uniformity test, disintegration time, and content variation range of phenolic compounds test. Tablets also underwent the Friability test using a friability tester and hardness tests using a hardness tester.

Figure 3: Mixed Powder and Compressed Tablets

Pre-formulation Studies of Formulated Tablet

The angle of repose was determined using the fixed funnel method. The angle of repose was calculated using the fixed height method to determine the flow characteristics of thoroughly mixed powder in the formulation. A funnel with a bottom diameter of 10 mm was positioned at a height of 2 cm above a plain and smooth surface. About 50 gm of a properly mixed sample was progressively moved alongside the funnel's wall until the pile's tip developed and touched the funnel's bottom. A rough circle was drawn around the pile's base, and the radius of the powder cone was measured, as well as the pile's height, using a capillary tube inserted into the pile. Finally, the height and radius of the resulting cone were measured and the angle of repose was calculated the formula,

Angle of repose (θ) = $\tan^{-1}(2H/D)$, Where, H=height of cone formed and D= diameter of the base.

In the compressibility Index, the known weight of the powder sample was weighed and gently poured into the 50 ml dry measuring cylinder. Note the initial fluffy volume as bulk volume Vo. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (V_o) to the nearest graduated unit. Calculate the bulk density in (g/ml) using the formula m/Vo. The measuring cylinder or vessel is mechanically tapped after the initial powder volume or mass is seen, and volume or mass readings are collected until little further volume or mass change is observed. Mechanical tapping is accomplished by elevating the cylinder or vessel and letting it descend a defined distance under its mass. Carry out 100 taps and after constant volume, tapped volume V_f is noted and tapped density was calculated. Finally, Carr's index was calculated by using formula:

Compressibity index = (ρbulk-ρtapped/ ρtapped) *100

Hausner ratio is the ratio of tapped density W/V_{50} to fluffy density (W/V_0 g/ml). A good flow is indicated by a Hausner ratio greater than 1.25, and a poor flow may have a value of 1.5. Hasuner ratio is calculated as the same procedure followed by Compressibility Index using the formula: Hausner Ratio = Tapped density/bulk density.

In-Vitro Studies of Formulated Tablet

The compressed tablets were examined for appearance and color. The tablets were examined by direct observation on a white paper background for the presence of any cracks, mottling, or using a magnified lens and other observations concerning the description of uncoated tablets. The thickness of the tablets was evaluated by Vernier Calipers. The weight variation of tablets was carried out to ensure that the tablets contained the proper amount of drug. The test was performed as per USP by weighing 20 tablets individually using an analytical balance, then calculating the average weight and comparing the individual tablet weights to the average. The weight variation test would be a satisfactory method of determining the drug content uniformity of tablets if the tablets were all or essentially all (90-95 %) active ingredients, or if the uniformity of the drug distribution in the granulation or powder from which the tablets were made perfect. In determining the disintegration time, from each formulation, 6 tablets were randomly selected. Each tablet was put into 900 ml HCl solution (0.1N) and the acidic buffer (pH 1.2) as a disintegration medium and the temperature was maintained at 37±0.5°C. The disintegration time of uncoated tablets was found to be 14 minutes. A Hardness Tester was used to determine the hardness of tablets. The tablet was placed between two anvils and force was applied to the anvils, and the crushing strength that caused the tablet to break was recorded. The crushing strength test was performed on 20 tablets of each formulation. The friability of tablets was determined using a Friability Tester. The dust particles present were removed before the test. If the tablet weight is 650 mg or less, then 6.5 grams of sample tablet weight was taken. If the tablet weight is greater than 650 mg, ten tablets of each formulation were weighed accurately and were tested at a speed of 25 rpm for 4 min (100 rotations). Any loose dust from the tablets was removed then weight the tablets again & calculate its friability.

% Loss Friability = Initial weight – Final Weight / Initial Weight * 100

Moisture content in powder was measured by a Moisture Analyzer instrument. It is used to determine how much moisture is present in a product.

Result

Yield of the plant

Yield is the amount or quantity produced by the *Urtica Dioica.* The result shows that ethyl acetate yield about 187 %, methanol (130%) and ethanol (89%) from Table 1. Ethyl acetate shows greater yield than other solvents which means that ethyl acetate produce more amount of antioxidant property than other. The yield percentage of the

plant extract in ethanol, methanol, and ethyl acetate was calculated. The yield percentage was calculated by using the following formula:

Optimization of extraction solvent

Methanolic extract was found good yield but rejected due to toxicity and we have to be more careful about pH. Ethyl acetate has the highest yield but didn't chosen as it extracted less amount of total tannin. Thus the ethanol was used for the extraction because it is more relevant and extracts good amount of polar compounds such as tannin and total phenolics.

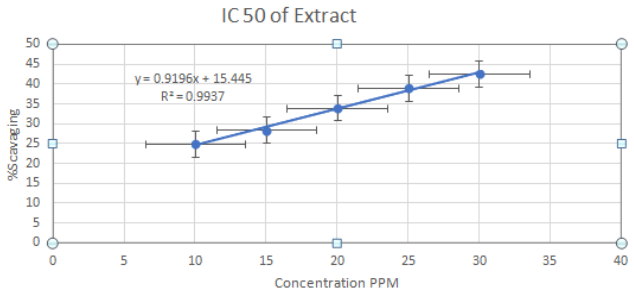

Figure 5: IC50 value of Plant Extract

Table 8: Determination of Total Phenolic Content

S. N	Concentration (ppm)	Absorbance of Standard	Absorbance of Sample
1	10	0.6976	1.2939
2	15	0.967	0.6033
3	20	1.2	0.3447
4	25	1.57	0.7408
5	30	1.733	0.9327

Table 6: Antioxidant property of Ascorbic Acid

S. N	Concentration ppm	Absorbance A°	% Scavenging	IC_{50} µg/ml
1	10	0.20	13.31	21.23
2	15	0.19	16.16	
3	20	0.13	42.91	
4	25	0.08	66.29	
5	30	0.03	86.94	

Table 7: Antioxidant property of *Utrica dioica* L. Leaves

S. N	Concentration ppm	Absorbance A°	% Scavenging	IC_{50} µg/ml
1	10	0.17	24.93	**37.57**
2	15	0.16	28.47	
3	20	0.15	34.05	
4	25	0.14	39.11	
5	30	0.23	42.61	

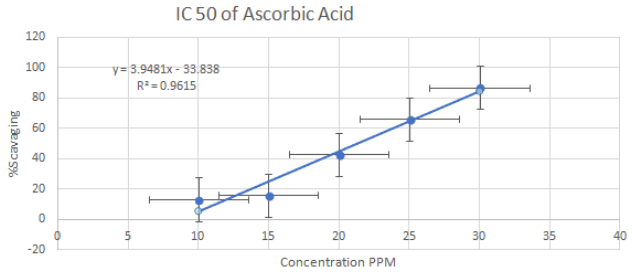

Figure 4: IC50 Value of Ascorbic Acid

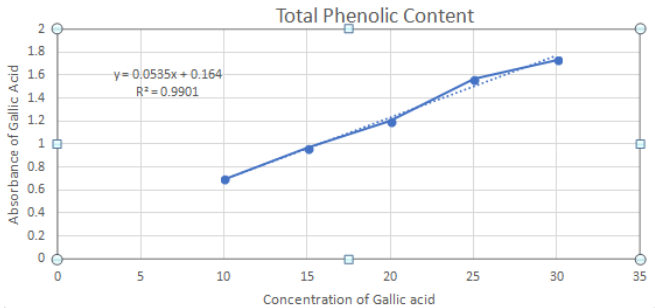

Figure 5: Calibration curve of Gallic acid for determination of Total Phenolic Content

Substituting the value of absorbance of sample in y, we obtained the Gallic acid concentration in the sample. Y = Absorbance, X = Gallic acid concentration in µg/ml, m = Slope, c = Constant,

X = Y – 0.164 / 0.0535

Table 9: Determination of Total Phenolic Content

Sample Solution (µg/ml) (V)	Weight of dry extract per ml in milligram (m)	GAE concentration (µg/ml) (c)	GAE concentration (mg/ml)	Total Phenolic as GAE C = c V/m (mg/g)	Mean
500	50	21.119	0.021119	211.19	
500	50	8.2112	0.0082112	82.112	**115.7146**
500	50	3.3776	0.0033776	33.776	
500	50	10.7813	0.0107813	107.813	
500	50	14.3682	0.0143682	143.682	

Content of Extracts

Total phenolic content in young leaves in ethanolic extract was found to be 115.7146 mg (Table 6) GAE/100gdw having the highest in 10 ppm concentration.

Evaluation of Prepared Powder and Compressed Tablet

The angle of repose was found to be 33.69˚ (good). The Compressibility Index was found to be 26.609 (poor). Hausner Ratio was found to be 1.363 (poor). Color Appearance was found to be light Green in color presence with break marks similar to as some research papers. The average thickness of 20 tablets was found to be 4.8 mm. The average Hardness of 20 tablets is 4.2 (kg/cm). % Loss Friability is 0.7 % which passes the criteria of not more than 1%. Disintegration time was found to be 14 minutes which passes the test. The prepared tablet consists of 4.57 % Moisture Content.

Since the total tablet weight is 300 mg, the % deviation for uncoated tablets is 7.5%. The range of weight variation (average weight ± 7.5% of average weight) is 288.6 mg to 335.4 mg. The total weight of 20 tablets is 6.240. The average weight of 20 tablets is 0.312 which falls within the acceptable weight variation range of ± 7.5%, hence, the tablets passed the weight variation test.

Discussion

The choice of solvent in herbal extract formulation plays a crucial role in determining the extract's composition and properties. While methanol is a common solvent in such studies, its potential toxicity prompted the use of dehydrated ethanol (100%) in this project. The comparative analysis of solvents revealed that ethyl acetate yielded the highest phenolic extract, surpassing both methanol and ethanol. However,

considerations such as polarity and toxicity led to the selection of ethanol for the extraction process.

Despite its lower yield, ethanol extraction, known for its non-toxic nature, aligns with safety concerns. The decision to focus on young leaves of Urtica dioica is supported by literature indicating superior antioxidant properties compared to mature leaves. Phenolic compounds, specifically those with OH groups, are recognized for their scavenging abilities, emphasizing the importance of herbal phenolic chemicals in contributing to antioxidant activity.

Quantitative assessments, such as the IC50 values, further support the antioxidant potential of the plant extracts. Ascorbic acid, a reference standard, exhibited a lower IC_{50} compared to the plant extracts. However, the plant extracts' IC50 values, especially considering the chosen phenolic-rich herbs, indicate their viability as readily available natural antioxidants. The finding was as in agreement with previous similar work [13]

Moving to the formulation aspect, the powder mixture's angle of repose of 33.69 signifies excellent flowability. In contrast, the compressibility index of 26.609 suggests poor compressibility, corroborated by a Hausner ratio of 1.363. These indices highlight challenges in powder compression, likely attributed to interparticle interactions.

The tablet characteristics, including disintegration time and hardness, meet relevant standards. The disintegration time of 14 minutes adheres to the 15-minute limit set by the Indian Pharmacopeia for uncoated tablets. Additionally, tablet hardness exceeding 3.5 confirms satisfactory mechanical strength, aligning with established benchmarks.

In summary, the solvent choice, plant part selection, and formulation parameters collectively contribute to the development of an herbal extract with promising antioxidant properties. The findings provide a foundation for further exploration and potential applications in the pharmaceutical or nutraceutical industries.

Conclusion

This study focused on formulating and assessing herbal antioxidant tablets derived from *Urtica dioica* L extracts for the treatment of arthritis and osteoarthritis. The antioxidant activity of the extract was confirmed with IC_{50} value of 37.57 µg/ml. The total phenolic content of extract was found to be 115.71 mg/g of the Gallic acid equivalent.

Herbal tablets were successfully formulated using the direct compression method, with a detailed evaluation of various formulations. The tablets demonstrated good flow properties, uniformity, appropriate hardness, and low friability. The study enhances our understanding of natural remedies and their therapeutic applications, offering a promising avenue for the development of herbal antioxidant tablets in the treatment of arthritis and osteoarthritis.

Acknowledgments

The research team is thankful to the Department of Pharmacy, Kathmandu University for providing the facilities for carrying out this research work.

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