

## Free Radical Scavenging Activity and Antioxidants of *Hydrocotyle vulgaris* L. (Pennywort): Baseline Study in Developing Biocosmetic-Antidote for Pathological Aging

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**Abstract.** Pathological aging due to harmful free radicals and oxidative stress has been a serious threat to human health. Thus, the study aimed to evaluate the free radical scavenging activity, antioxidants, essential vitamins, and physicochemical properties of *Hydrocotyle vulgaris* L. (Pennywort) which could serve as basis in developing a biocosmetic antidote for pathological aging. *H. vulgaris* L. plant was harvested, air dried and then extracted via soxhlet. UV-vis spectroscopy was used for scavenging activity and antioxidants of the plant while High Performance Liquid Chromatography (HPLC) was applied for the identification of essential vitamins. Phytochemicals, pH and spreadability values of the plant were also tested. Results revealed that *H. vulgaris* L. has an abundant presence of flavonoid and alkaloid while traced presence of tannin. Good trend for scavenging activity was also observed exhibiting low absorbance of possible free radicals with IC<sub>50</sub> of 29.75 and antioxidant activity of 158.13 (Total Phenolics as gallic acid in %w/w). Meanwhile, β-carotene (10.4 mg/kg), Riboflavin (4.08mg/kg), and Vitamins C (70.2mg/kg) and E (26.9mg/kg) were also found in the plant. Non-irritating pH levels of 5.7 and 7.14 for 50% and 75% concentrations of the extracts with good spreadability value of 17.51 g·cm/sec (for 75% concentration) were also obtained. Based on the findings of the study, Pennywort has a very promising therapeutic characteristics with its good scavenging activity and antioxidants. Thus, the development of a biocosmetic product that could serve as an antidote for pathological aging from Pennywort appeared to be very apparent.

### Introduction

The National Institutes of Health (NIH) recognizes 20–30 different theories of aging, the most widely accepted of which is the Free Radical Theory. This theory was first developed by Denham Harman and has been discussed for more than 50 years. The Free Radical Theory states that accumulated free radical damage and oxidative stress alter biochemical and cellular processes as aging damage accumulates. Most free radical damage occurs during times of active metabolic turnover. In humans, this occurs in early puberty for males and in pre-puberty and early puberty for females. Also during this time, humans possess the most physiologic reserve. However, as damage accumulates, our physiologic reserve becomes depleted [4].

The human skin, being the body's initial environmental protector, is exposed to other sources of free radical damage in addition to sun and internal cellular metabolism. Other sources of free radical damage to the skin include ozone, pollutants, applied materials (some commercial sunscreens), alcohol, severe physical and emotional stress, poor nutrition, obesity, and toxins. Smoking is also critically harmful to cells and tissues, by delivering massive amounts of free radicals with every puff.

Today there are so many available products in the market that suggest solution on the problems brought by oxidative stress and free radicals. But most of these products are not totally helping but causing additional damages on the skin instead.

DeHaven [4] also argued that there are mechanisms for neutralizing free radicals, such as antioxidants. Antioxidants absorb free radicals and stop the negative cascade of molecular damage. There are lipid-soluble antioxidants, such as vitamin E, which target the lipid-rich or fat-containing parts of cells. There are aqueous antioxidants, such as vitamin C, which protect the water-containing -fluid portions of cells. Extrinsic antioxidants are ingested or applied topically, whereas intrinsic antioxidants are present inside cells and are synthesized by the body. Intrinsic antioxidants include enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes are manufactured within the interior of the cell, as an inherent defense against free radical damage.

Various researches had been conducted using different plants that are rich in phytonutrients and phytochemicals that could act as antioxidants that will hinder effects of free radicals. Natural-utilizing researches are more valued because of its natural and low risk effects on humans.

In the present study, *Hydrocotyle vulgaris* L. (Pennywort) plant will then be subjected to preliminary analyses regarding its free radical scavenging activity, antioxidant capacity, essential vitamins and other physicochemical properties that will serve as baseline for the development of new cosmetic products that will be an antidote for pathological aging.

## Materials and Methods

### Plant material preparations

Plant material *Hydrocotyle vulgaris* L. (Pennywort) was harvested from Bansud Oriental Mindoro. The plant's leaves and stems were cleaned, and placed on a screen for air drying (depending on the condition of the plant and the temperature).

Meanwhile, live plant sample of *Hydrocotyle vulgaris* L. (Pennywort) was sent to the National Museum for documented certification of identification.

### Sample Preparations

After the air drying process, the plant material was powdered using mechanical grinding mill obtaining 3 kilograms of powder. From this, 2 kilograms was set aside as sample for standardized laboratory testing of *Hydrocotyle vulgaris* L. (Pennywort).

### Soxhlet assisted extraction

The powders of samples were extracted exhaustively in a Soxhlet using water as solvent. The aqueous extract was kept in a well closed container in refrigerator until use [5].

### Plant material standardized testing

#### *Free radical scavenging activity and antioxidants of the plant material*

Two (2) kilograms of dried powdered *Hydrocotyle vulgaris* L. (Pennywort) was extracted using soxhlet (aqueous) and subjected to standardize testing for free radical scavenging activity and antioxidant capacity at the Department of Science and Technology – Industrial Technology Development Institute (DOST-ITDI) using UV-Visible Spectrophotometer. The result for the antioxidant activity (Total Phenolic) was expressed in Gallic Acid Equivalent (GAE) applying the formula  $GAE = c \times V/M$  where  $c$  was the concentration,  $V$  was the volume used during the assay and  $M$  as the mass of the extract used.

Ten (10) sample preparations were done for the radical scavenging activity with ten (10) different concentrations such as 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50  $\mu\text{g/ml}$ .

The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ scavenging} = [\text{Absorbance of control} - \text{Absorbance of test sample} / \text{Absorbance of control}] \times 100$$

The effective concentration of sample required to scavenge DPPH radical by 50% (IC<sub>50</sub> value) was obtained by linear regression analysis of dose-response curve plotting between the % of DPPH radical scavenging activity and concentrations (Iranshahi et al., 2009). The IC<sub>50</sub> ("efficient

concentration" value) was used for the interpretation of the results from DPPH method. The  $IC_{50}$  was obtained from the equation of the curve  $y = 1.8719x - 5.6293$  with  $R^2 = 0.8653$ .

#### *Determination of necessary vitamins (HPLC) and phytochemical constituents*

From the initial two (2) kilogram-powdered samples of the *Hydrocotyle vulgaris* L. (Pennywort) that was extracted (aqueous) via soxhlet, High Performance Liquid Chromatography (HPLC) was applied in determining the amount (in mg/kg) of the essential vitamins like Vitamin B2 (Riboflavin), Beta-Carotene and Vitamins E and C.

On the other hand, the presence of various phytochemical constituents was also examined particularly the flavonoid content of the plant material. The testing was administered at the Department of Science and Technology – Industrial Technology Development Institute (DOST-ITDI).

#### *Evaluation of physicochemical parameters*

The physicochemical properties such as pH and spreadability of the aqueous extracts of the *Hydrocotyle vulgaris* L. (Pennywort) were evaluated.

#### *pH testing*

20 g of the aqueous extract of the plant material mixed with a lotion-based cream with different concentrations (25%, 50% and 75%) was dispersed in 80 ml of distilled water to determine the pH at 27°C using the pH meter.

#### *Spreadability testing*

In the spreadability testing of *Hydrocotyle vulgaris* L. (Pennywort), its extracts were mixed to a lotion-based cream with 25%, 50% and 75% concentrations.

The parallel plate method was applied in determining the spreadability of semisolid preparations. The setup was consisted of two glass slides placed on a tripod stand on which excess of cream (3 g) was applied in between of the two glass slides. The upper slide was movable and the lower slide was firmly fixed to the stand. 100 g weight was placed on them for 5 minutes to compress the cream to uniform thickness and the excess cream had was scrapped off from the edges. Then 50 g weight was added to one side of the slide and the slide was pulled until a distance of 10 cm was covered. The time in seconds to separate two glass slides by 10 cm and this was taken as a measure of spreadability [8]. A shorter interval indicated better spreadability [13].

The spreadability was calculated by using the formula,  $S = m.l/t$  where, S=Spreadability, m=Weight tied to upper glass slide, l=Length of glass slide, t=Time taken to separate them.

#### *Data analysis*

One-factor Analysis of Variance and Mean were used in the study particularly in the comparisons and differences on the physicochemical analysis of the extracts of the plant material.

## Results and Discussion

**Table 1.** Result of Phytochemical Constituent Testing for *Hydrocotyle vulgaris* L. (Pennywort)

Phytoconstituent	Result
Sterols	(++)
Triterpenes	(+)
Flavonoids	(+++)
Alkaloids	(+++)
Saponins	(++)
Glycosides	(+)
Tannins	(+)

Note: (+) Traces, (++) moderate, (+++) abundant (-) absence of constituents

Reference: Pharmacology, 15<sup>th</sup> edition, 2002, Trease & Evans

Results revealed the presence of constituents such as sterols, triterpenes, flavonoids, alkaloids, 4aponins, glycosides and tannins in *Hydrocotyle vulgaris* L. (Pennywort) plant extracts as shown in Table 1.

It can also be deduced from the above table that *Hydrocotyle vulgaris* L. (Pennywort) has trace presence of triterpenes, glycosides and tannins, while there is a moderate presence of sterols and 4aponins and an abundant presence of alkaloids and flavonoids.

Among the phytoconstituents present on the plant material, flavonoids and tannins have shown to have antioxidant activity. The abundance of the flavonoid assures greater capacity of antioxidant activity to protect human cells against oxidative damage and reduce the risk of developing certain types of cancer.

According to Corradini et al. [19], flavonoid is a general name of a class of more than 6500 molecules based upon a 15-carbon skeleton. The core structure is a 2-phenylbenzopyranone, in which the three carbon bridge between the phenyl groups is commonly cyclised with oxygen. Therefore flavonoids have been recognised as one of the largest and most widespread groups of plant secondary metabolites with marked antioxidant properties. From this, the *Hydrocotyle vulgaris* L. (Pennywort) could be considered as a plant with good antioxidant capability since it demonstrated an abundant presence of flavonoids.

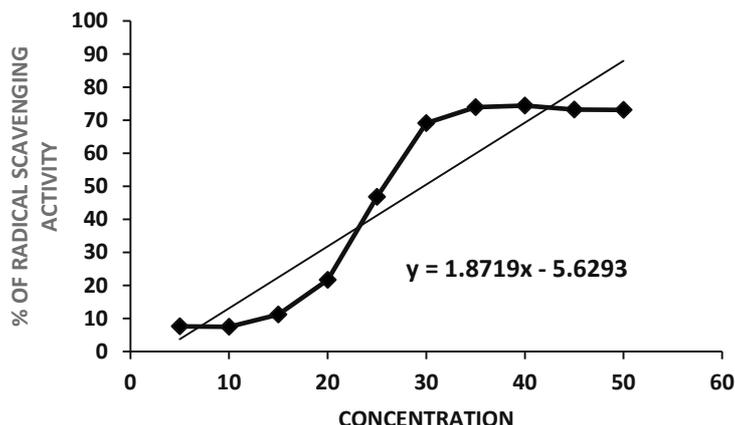
Meanwhile Gong et al., (2010) argued that an active flavonoid compound possesses potent antioxidant properties against oxidative stress. This assured the capability of the *Hydrocotyle vulgaris* L. (Pennywort) to fight oxidative stress that can cause cell damage.

The abundance of alkaloids on the other hand appeared to be directly associated with the antioxidant capacity of the *Hydrocotyle vulgaris* L. (Pennywort), this is based on the conducted study of Maiza-Benabdesselam et al. [10] wherein both alkaloid extracts of the two species of *Fumaria* showed a strong antioxidant activity, especially a strong radical scavenger power, so they can be used as natural and good sources of natural antioxidants for medicinal and commercial needs.

In the study undertaken by Hagerman [6], although dietary tannins are often perceived as detrimental because of their potential to affect protein digestibility or metal ion availability, it is also possible that tannins are beneficial. It is likely, based on the knowledge of tannin chemistry that tannins are potential biological antioxidants.

**Table 2.** Characteristic of the *Hydrocotyle vulgaris* L. (Pennywort) extracts in terms of diphenylpicrylhydrazyl (DPPH) Free Radical Scavenging Activity

Sample Preparation No.	Concentration	DPPH Radical Scavenging Activity (%)	IC <sub>50</sub>
1	5	7.61	
2	10	7.48	
3	15	11.2	
4	20	21.7	
5	25	46.8	
6	30	69.1	<b>29.75</b>
7	35	73.9	
8	40	74.4	
9	45	73.2	
10	50	73.1	



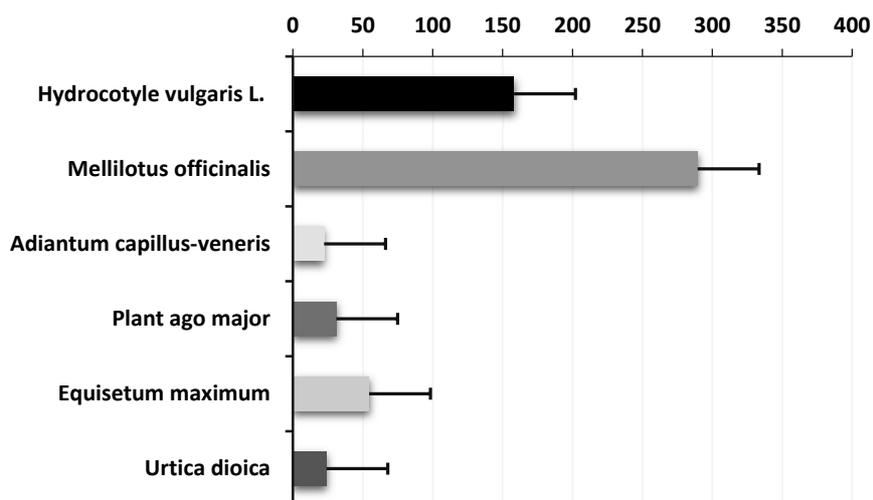
**Figure 1.** Graphical presentation of % of DPPH Radical Scavenging Activity

Unlike other free radicals such as the hydroxyl radical and super oxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition [1]. The readings of DPPH radical scavenging activity (in %) showed a slight direct proportionality since the last two readings demonstrated a slight decline as shown in Table 2 and Fig. 1. But then, according to [20] this behaviour of the percentage of scavenging activity using DPPH is normal because as the concentrations continue to increase there will be still a decrease in the absorbance or conversion of the colour medium to colourless. This happened because the antioxidant molecules can reduce DPPH free radicals by providing hydrogen atoms or by electrons donation, conceivably via free radical attack on the DPPH molecule.

On the other hand, the decrease in the absorbance was evident through the exhibited percentage of DPPH free radical scavenging activity presented at Table 2 since the results showed increase and this is actually inversely proportional to the absorbance [20]. Hence, the more rapidly the absorbance decreases (or % of scavenging activity increases), the more potent commonly employed assay in antioxidant studies of specific compounds or extracts across a short time scale.

Shown also in Table 2 is the  $IC_{50}$  value of the *Hydrocotyle vulgaris* L. (Pennywort) which was 29.75. This result was closer to the presented  $IC_{50}$  value of the ascorbic acid in the study of M. Anil and P. Suresh [20] which was 20.693. This was a good sign for a better performance on antioxidants since the obtained value was quite comparable with that of standard ascorbic acid.

In the study [21] the ethanolic extract of leaves of *Caesalpinia pulcherrima* showed  $IC_{50}$  value of 7.46 while the ethanolic extract of leaves of *Xanthium indicum* showed  $IC_{50}$  value of 90.43. They explained that *Caesalpinia pulcherrima* has a better antioxidant capacity. Maisuthisakul, Suttajit and Pongsawatmanit [22] furtherly stressed that the lower the  $IC_{50}$  value – the higher the antioxidant activity of the extracts. The obtained  $IC_{50}$  for the *Hydrocotyle vulgaris* L. (Pennywort) was at low value. This means that the presented results at Table 2 and Fig. 1 proved that the exhibited potent of increasing points on the DPPH scavenging activity lead to good  $IC_{50}$  value (29.75).



**Figure 2.** Tested Antioxidant Activity (Total Phenolics) of the *Hydrocotyle vulgaris L.* (Pennywort) extracts as compared with the Phenolics in extracts of different plants cited at [23]

As shown in Fig. 2, the result of antioxidant activity (Total Phenolics) of the *Hydrocotyle vulgaris L.* (Pennywort) extracts was 158.13. It was then compared with the Total Phenolics of different plant extracts in the study of Pourmorad, Hosseinimehr and Shahabimajd [23].

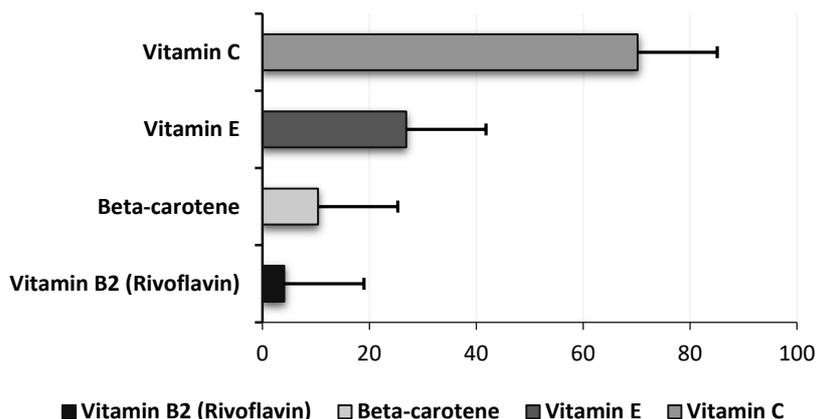
According to [25] and [18] as cited by Stanković [24] the total phenolic contents in plant extracts depends on the type of extract, i.e. the polarity of solvent used in extraction. In the current study, methanol was used. This was the same with the solvent used in evaluating the *Mellilotus officinalis*.

Based on the presented result the amount of Total Phenolics of *Hydrocotyle vulgaris L.* (Pennywort) appeared to relatively closer with the Phenolics of *Mellilotus officinalis* which was 289.5 which had the highest amount of Total Phenolics. Meanwhile Phenolics of *Hydrocotyle vulgaris L.* also appeared higher compared with the other four plant subjects such as *Adiantum capillus-veneris*, *Plant ago majo*, *Equisetum maximum*, and *Urtica dioica* which had 22.3, 31, 54.5 and 24 as Total Phenolics respectively.

It can be implied based on the presented result that *Hydrocotyle vulgaris L.* (Pennywort) had a high antioxidant capacity since plants with high amounts of Total Phenolics like *Mellilotus officinalis* are considered as good source of antioxidants. The greater amount of phenolic compounds leads to more potent radical scavenging effect [23].

The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants [3], [17]. The high contents (+++) of these phytochemicals in *Hydrocotyle vulgaris L.* can explain its high radical scavenging activity that led to high antioxidant activity.

According to Pietta [11] one of the many phenolic substances is flavonoid from a wide range of vascular plants, with over 8000 individual compounds known. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and for light screening. In the current study flavonoid first appeared to be abundant in *Hydrocotyle vulgaris L.* (Pennywort). This evidence of high Phenolics in *Hydrocotyle vulgaris L.* (Pennywort) and abundance in flavonoids gave implications that the plant material could exhibit good skin protection fighting oxidative stress that causes pathological aging.



**Figure 3.** Phytonutrient characteristic of the *Hydrocotyle vulgaris* L. (Pennywort) extracts in terms of like  $\beta$ -carotene, Riboflavin, Retinol, Vitamin C and Vitamin E

The phytonutrient characteristics of the *Hydrocotyle vulgaris* L. (Pennywort) extracts in terms of like  $\beta$ -carotene, Riboflavin, Retinol, Vitamin C and Vitamin E are shown in Fig. 3.

### Vitamin E

According to the National Institute of Health (2016) Vitamin E is a fat-soluble nutrient found in many foods. In the body, it acts as an antioxidant, helping to protect cells from the damage caused by free radicals. Free radicals are compounds formed when our bodies convert the food we eat into energy. People are also exposed to free radicals in the environment from cigarette smoke, air pollution, and ultraviolet light from the sun. An adult individual needs at least 15mg of Vitamin E in a day and based on the results of standardized test conducted via High Performance Liquid Chromatography (HPLC) a value of 26.9 mg of Vitamin E was obtained for *Hydrocotyle vulgaris* L. (Pennywort).

The Vitamin E present in *Hydrocotyle vulgaris* L. (Pennywort) implies a good amount to help the body fight free radicals and UV rays for the skin to recover from oxidation stress. The body also needs vitamin E to boost its immune system so that it can fight off invading bacteria and viruses. It helps to widen blood vessels and keep blood from clotting within them. In addition, cells use vitamin E to interact with each other and to carry out many important functions (NIH, 2016).

Kiefer (2015) furtherly argued that Vitamin E is the key for strong immunity and healthy skin and eyes. In recent years, vitamin E supplements have become popular as antioxidants. These are substances that protect cells from damage. This simply shows the significance of the said phytonutrient on addressing problems on skin and pathological aging.

### Vitamin C

Based on the several reviews conducted by Weil [26] Vitamin C (also known as ascorbic acid) is abundant in vegetables and fruits. A water-soluble vitamin and powerful antioxidant, it helps the body form and maintain connective tissue, including bones, blood vessels, and skin.

As shown in Fig. 3, *Hydrocotyle vulgaris* L. (Pennywort) had 70.2 mg of ascorbic acid.

The result was closer to the required dosages of Vitamin C for adult men and women which are 90 and 75 mg per day and more than enough for female teens (14-18y/o) which has only 65 mg requirement of intake in a day. Meanwhile the result was also closer to the 75 mg intake dosage/day for male teens (14-18y/o) [26].

In addition, in his reviews, Weil [26] also stressed that Vitamin C helps to repair and regenerate tissues, protect against heart disease, aid in the absorption of iron, prevent scurvy, and decrease total and LDL (“bad”) cholesterol and triglycerides. Research indicates that vitamin C may help protect against a variety of cancers by combating free radicals, and helping neutralize the effects of nitrites (preservatives found in some packaged foods that may raise the risk of certain forms of cancer). Supplemental vitamin C may also lessen the duration and symptoms of a common cold; help delay or prevent cataracts; and support healthy immune function.

As mentioned on the reviews above, ascorbic acid plays a vital role in shielding the body from the effects of free radicals that damages the skin causing it to age abnormally. So there is a clear good impact on the presence of Vitamin C in *Hydrocotyle vulgaris* L. (Pennywort) since it is considered as a powerful antioxidant.

### **Beta-Carotene**

Adults and teenagers have a dosage requirement of 6 to 15 milligrams (mg) of beta-carotene (the equivalent of 10,000 to 25,000 Units of vitamin A activity) per day as dietary supplement (Mayo Clinic, 2016). Meanwhile, the resulted amount of beta-carotene present in *Hydrocotyle vulgaris* L. (Pennywort) was 10.4 mg as shown in Fig. 3.

According to the article of Nordqvist [28] beta-carotene, like all carotenoids, is an antioxidant. An antioxidant is a substance that inhibits the oxidation of other molecules; it protects the body from free radicals. These free radicals damage cells through oxidation. Eventually, the damage caused by free radicals can cause several chronic illnesses. Several studies have shown that antioxidants through diet help people's immune systems, protect against free radicals, and lower the risk of developing cancer and heart disease. Some studies have suggested that those who consume at least four daily servings of beta-carotene rich fruits and/or vegetables have a lower risk of developing cancer or heart disease. Beta-carotene might help older people retain their lung strength as they age.

Schagen et al. [15] showed that the findings of Scarmo et al. suggest that human skin, is relatively enriched in lycopene and  $\beta$ -carotene, compared with lutein and zeaxanthin, possibly reflecting a specific function of hydrocarbon carotenoids in human skin photoprotection.

The amount of beta-carotene in *Hydrocotyle vulgaris* L. (Pennywort) was more than enough to cater the necessary dosage requirement a day. This gives a good implications that the plant material tend to be more rich and helpful in addressing pathological aging due to the effects of radiations from UV-rays and oxidative stress.

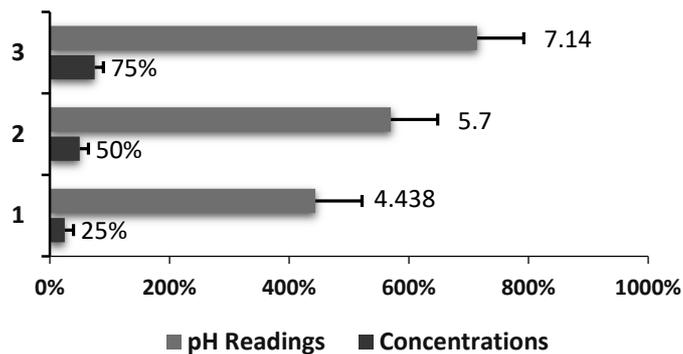
### **Vitamin B<sub>2</sub> (Riboflavin)**

Bradford (2015) stressed that Vitamin B<sub>2</sub>, also known as riboflavin, is one of the eight B-complex vitamins. Like other B vitamins, it plays a role in energy production in the body, but also has many other important uses. Vitamin B<sub>2</sub> is a water-soluble vitamin that is flushed out of the body daily, so it must be restored each day. The best way to get this vitamin is by eating foods that are rich in riboflavin.

As shown in Fig. 3, *Hydrocotyle vulgaris* L. (Pennywort) obtained 4.08 mg of Riboflavin. The presented result was actually higher on the Recommended Dietary Allowance (RDA) per day that was written on the review of University of Maryland Medical Center (UMMC) (2016) wherein adult men and women were recommended to have 1.3 and 1.1 mg of Riboflavin respectively. This means that the plant material, *Hydrocotyle vulgaris* L. (Pennywort) could really cater and provide the necessary Vitamin B<sub>2</sub> that the human body requires to address radical effects.

According to Worldhealth.net (2016) Vitamin B<sub>2</sub> is vital for the formation of FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide), both of which are essential for metabolising carbohydrates, proteins, and fats and make energy available in the body. The vitamin is also important to maintain metabolism and for the health and proper functioning of the cardiovascular and nervous systems. It also protects against free radical damage and is necessary for good vision, skin, hair, and nails. Physical exercise increases the body's need for vitamin B<sub>2</sub>. Research also suggests that vitamin B<sub>2</sub> may help to prevent or slow the development of cataracts, and reduce the frequency of migraines.

Ashoori and Saedisomeolia [27] explained in their study "Riboflavin (vitamin B<sub>2</sub>) and oxidative stress: A review" the confirmation of antioxidant nature of riboflavin and indicated that this vitamin can protect the body against oxidative stress, especially lipid peroxidation and reperfusion oxidative injury. The mechanisms by which riboflavin protects the body against oxidative stress may be attributed to the glutathione redox cycle and also to other possible mechanisms such as the conversion of reduced riboflavin to the oxidised form.



**Figure 4.** Graphical presentation of the pH readings of the extracts of *Hydrocotyle vulgaris* L. (Pennywort) in three different concentrations

Monitoring the pH value is important for determining the stability of pharmaceuticals and cosmaceuticals. Any change in pH of the product indicates a possible interaction or occurrence of chemical reactions which may provide an idea on the quality of the possible product [16].

According to Imam, Azhar and Mahmood [8], the pH of human skin normally ranges from 4.5 to 6.0. Because of the frequent washing and used of soap, the acidity of the skin is lost. Therefore, moisturizer has an acidic range should be used to normalize the skin.

As shown in Fig. 4, the pH readings of the extracts of *Hydrocotyle vulgaris* L. (Pennywort) were 7.14, 5.7, and 4.438 for 75%, 50%, and 25% of concentrations respectively. Based on the study of Saraf, Sahu and Kaur [14], the acceptable pH range of moisturizers should be 5-8. The extracts of *Hydrocotyle vulgaris* L. (Pennywort) with 75% and 50% concentrations had 7.14 and 5.7 which are acceptable pH readings and considered as non-skin irritating pH value.

The results also revealed a directly proportional relational relationship between the concentrations and pH readings. This furtherly explained that the higher concentrations could result to an even higher pH results.

**Table 3.** Characteristic of the *Hydrocotyle vulgaris* L. (Pennywort) extracts in terms of spreadability value (SV)

Parameter: Spreadability	
Concentrations	SV
25%	11.33±1.25
50%	15.05±1.76
75%	17.51±0.71

Notation: SV=Spreadability Value

Values are given in mean±SD within five trials of testing

The results of the spreadability value of *Hydrocotyle vulgaris* L. (Pennywort) are shown in Table 3. The results are based on parallel plate method conducted during the experimentations applying three different concentrations such as 25%, 50% and 75%.

Spreadability is a term expressed to denote the extent of the area to which the topical application spreads on affected parts of the skin. The therapeutic efficacy of the formulation depends on its spreading value [8].

Based on the presented results, it was 75% concentration which obtained the highest value of spreadability which was 17.51, followed by the 50% with 15.05 and 11.33 was gained by the 25% of concentrations being the lowest SV computed value.

According to the study of R.K. Purushotham et al. [13] a shorter interval indicates better spreadability. This was aligned with the results presented in the current study wherein the 75% of concentration had the shortest time interval (refer on the appendix) which obtained the highest value of spreadability.

In addition, the result of the SV of the current study is closer to the result presented by Imam, Azhar and Mahmood [8] which was  $17.25 \pm 0.35$  particularly the SV in the 75% concentration which was also a little bit higher.

**Table 4.** Difference on the characteristic of the *Hydrocotyle vulgaris* L. (Pennywort) extracts in terms of spreadability value (SV) applying various concentrations

Concentrations	Mean	n	Std. Dev	df	SS	MS	F	p-value	Interpretation
25%	11.33	5	1.25	4	96.80	48.40	28.28	2.88E-05	Significant
50%	15.05	5	1.76	4					
75%	17.51	5	0.71	4					

As shown in Table 4, there is a significance difference on the calculated spreadability values for the various prepared concentrations such as 25%, 50% and 75% with the computed p-value of  $2.88E-05$  which was lower than 0.05 level of significance.

Based on the presented results, it can then be implied that the interval (time in seconds) in separating or pulling the two slides in parallel plate method for spreadability calculations depends on the amount of extracts of the plant.

It can also be deduced on the result that the higher concentrations of the extract the lower time interval will be recorded that would lead to higher value of spreadability.

## Conclusion

The study provided reasonable data to conclude that *Hydrocotyle vulgaris* L. (Pennywort) possesses good free radical scavenging activity with  $IC_{50}$  value of 29.75 which proved its high antioxidant property of 158.13 which was capable of protecting the skin from the harmful effect of various physico-chemical factors and oxidative stress. In addition, important vitamins like  $\beta$ -carotene, Riboflavin, Vitamin C and Vitamin E in *Hydrocotyle vulgaris* L. appeared to be very evident with amounts of 10.4, 4.08, 70.2, and 26.9 mg/kg respectively. Abundant presence of flavonoids, alkaloids and traced appearance of tannins was also observed showing its direct connection to the occurrence of good antioxidant activities. *Hydrocotyle vulgaris* L. also possessed a very promising pH results which falls at the required pH readings of moisturizers. On the other hand, good spreadability value of 17.51 (for 75% concentration) was also obtained from the extracts of the plant subject.

The results of the current study give strong implications that *Hydrocotyle vulgaris* L. (Pennywort) could give possible effects and help the human skin to be protected. On the other hand, upon screening the *Hydrocotyle vulgaris* L. (Pennywort) plant, the possibility of the development of biocosmetic products from it appeared to be very apparent. This could really help to fight harmful free radicals and oxidative stress. Therefore, *Hydrocotyle vulgaris* L. (Pennywort) could serve as an antidote for pathological aging.

## Conflict of Interest

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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