

# Phytochemical Screening and Antioxidant Analysis of the Ethanolic Extract of Rosehip Seed Press Cake

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## Authors' contributions

This work was carried out in collaboration among all the authors. Author EBT designed the study and guided the laboratory analysis. Author RPM did the practical work in the laboratory and contributed to the literature search and writing of the first draft of the manuscript. Author IH supervised the laboratory study, contributed to literature search, data analysis and writing of the first draft of the manuscript. Author SM provided the methods for DDPH free radical scavenging assay and guided laboratory work on the phytochemical screening of the different classes of phytochemicals and total phenolic content and the antioxidant scavenging analysis. All the authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** Rosehip seed press cake is a waste material in the industrial extraction of rosehip seed oil using cold press process. The current study seeks to evaluate the phytochemical profile, total phenolic content, total flavonoid content and DPPH radical scavenging activity of the ethanolic extract of rosehip seed press cake using standard methods and DPPH assay.

**Study Design:** This is an experimental laboratory report on the phytochemical properties and antioxidant potential of the ethanolic semi-solid extract (ESE) of rosehip seed press cake in order to assess its commercial viability as a food supplement.

**Place and Duration of Study:** The work was conducted in the Department of Chemistry, National University of Lesotho, from August 2019 to March 2020.

**Methodology:** Ethanol was used for the extraction of the semi-solid extract (ESE) from the rosehip seed press cake. The ESE was analysed for phytochemical constituents using standard methods. *In*

*vitro* antioxidant activity of the ESE was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. For data analysis, mean and standard deviation were computed for each parameter that was quantitatively determined.

**Results:** Presence of flavonoids, alkaloids, terpenoids, sterols, glycosides, reducing sugars, proteins, amino acids, fatty acids, phenols and polyphenols in the ESE was confirmed. Total phenolics and flavonoids content were found to be 134.44 mg GAE/g DW and 73.23 mg QE/g DW, in the range of 200 to 3000 µg/ml respectively. The DPPH radical scavenging activity of the extract was found to be in the range 10.32±3.89 and 76.06±3.48% within the concentration range and was very close to the scavenging activity of DPPH at concentrations ≥ 1500 µg/ml. The ESE showed an IC<sub>50</sub> value of 1367.06 µg/ml relative to that of the positive control, ascorbic acid, being <200 µg/ml.

**Conclusion:** The ESE from the rosehip seed press cake was found to be very promising as a food supplement since 50% of the 16 screened phyto-constituents were present in high concentrations; 6.25% were detected in moderate concentrations and another 6.25% were present in low concentrations. Furthermore, the ESE exhibited antioxidant properties. Further studies are recommended to obtain more information on its composition and suitability additive in animal feed or as a food supplement.

**Keywords:** Antioxidant activity; ethanolic extract; press cake; DPPH assay; Folin-Ciocalteu assay.

## 1. INTRODUCTION

In recent years, medicinal plants have gradually been gaining global recognition and significance owing to their bioactive phytochemical constituents. Medicinal plants are rich in secondary metabolites, which in turn are potential natural sources of novel drugs [1,2]. Typical secondary metabolites with significant pharmacological and therapeutic properties include alkaloids, anthraquinones, flavonoids, glycosides, phenolics, saponins and tannins [3, 4]. Phenolics are also known to display a wider range of biochemical properties such as antioxidant activity, anti-mutagenesis, and anti-carcinogenesis [5]. Antioxidants are chemical substances that inhibit oxidative chain reactions which occur when free radicals are generated within cells and cellular membranes [6]. Some of the potent antioxidants include carotenoids, vitamins C and E [3]. Thus, antioxidants protect human cells against the negative effects of free radicals, which include heart diseases and cancers.

Rosehips, which are pseudo fruits of *Rosa canina* have been reported to be rich in carotenoids, vitamin C, phenolics, lycopene and other bioactive phyto-constituents [5,7]. The rosehip or wild rose, *Rosa canina* L., commonly known as dog rose, belongs to the Rosaceae family in the genus *Rosa* and is a wild shrub widely reported as indigenous to Europe [5,7,8]. Rosehip is largely explored for its prophylactic and therapeutic potential to treat ailments such as urinary tract infections (UTIs), common cold, inflammatory diseases, infectious diseases and

gastrointestinal disorders [9,10]. Currently, rosehip fruits are used in various parts of the world with a variety of functional focuses. For instance, in Lesotho, rosehip fruits are mainly used to make rosehip juice and tea blends and extraction of the oil. Rosehip seed oil finds a wide application in cosmetics. The nutritional content of rose hips is also reported to be relatively high. Some researchers have reported 100 g of rose hips to contain 162 kcal energy, 38.22 g carbohydrates, 1.6 g protein, 0.34 g fat (total lipid), 24.1 g fibre, 2.58 g sugars, 426 mg vitamin C (total ascorbic acid), 1.3mg niacin, 0.076 mg vitamin B-6, 0.166 mg riboflavin, 5.84 mg vitamin E and 4345UI vitamin A (UI) [5].

The exploitation of rosehip seed oil for commercial purposes has become the focus of many researchers globally. Various studies have shown that rosehip seed oil is susceptible to degradation at high temperatures. Thus, rosehip seed oil extraction methods involve proper control of temperature in order to ensure high quality oil. Therefore the rosehip seed oil extraction is commonly carried out by the cold press process. However, whichever method is used the extraction process generates various by-products, including the solid rosehip seed press cake. The seed press cake generated at the rosehip processing plant in Lesotho, which is the only one in the country, is used as feedstock for horses and it is sold to local farmers for use for that purpose. The literature search by authors revealed that the press cake generated in Lesotho has not been explored for its phytochemical and therapeutic properties. The current study aimed to bridge that information

gap by evaluating the qualitative phytochemical profile, the total phenolics and flavonoids content and the *in vitro* antioxidant activity, of the ethanolic semi-solid extract from the rosehip seed press cake obtained from rosehip oil extraction plant in Lesotho.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

The chemicals ascorbic acid, sodium hydroxide, aluminium chloride, ferric chloride, copper sulphate and potassium ferricyanide were all AR grade and purchased from Sigma-Aldrich through a local supplier, Prestige Laboratory Supplies. The other chemicals ethanol (99%), methanol (99%), concentrated sulphuric acid (98%), concentrated hydrochloric acid (32%), and magnesium powder were all AR grade, acetone (CP grade) and the reagents diethyl ether, Ninhydrin, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), quercetin, Biuret's reagent, Fehling's solution and Benedict's reagent were CP grade and were purchased from Sigma-Aldrich through a local supplier, Minema Chemicals.

### 2.2 Material Collection

A bulk sample of press cake pellets was obtained from the rosehip processing plant at the Rosehip Company, which extracts the rosehip oil in Mophale's Hoek, Lesotho (coordinates: 29°30'S 28°30'E, 29.500°S). Lesotho is a small country entirely landlocked by South Africa and has four agro-ecological zones, the mountains, foot-hills, lowlands and Orange-River-Valley. The rosehip plant is not confined to any of these four agro-ecological zones, but it grows throughout the country. However, it is found in relatively greater amounts in the highlands and foothills where the climate is cooler and annual rainfalls are higher than the other two zones.

The processing plant gets its raw materials, the rosehip fruit from numerous small scale contractors who harvest the fruits and sell them to the company. The widely distributed rosehip species found in Lesotho is *Rosa canina* L with a small scale distribution of the *Rosa rubiginosa* species hence the rosehip cake press is a mixture of the two species.

### 2.3 Material Processing

An amount of about 1kg of the cake sample was ground into a fine powder using a hand-screw

mill and the powdered sample was stored in a clean sample bag from which the required amount was drawn for specific investigations.

### 2.4 Extraction of Ethanolic Semi-Solid Extract

A 500-ml round bottom flask was used to carry out maceration at room temperature. The process involved mixing 500. g of the powdered press cake with 800 mL of 99% ethanol and holding the mixture for three days (72 hours) with occasional shaking. The extract solution was then filtered off using a vacuum pump (ATB Model 284065-H). The ethanol solvent was removed using Büchi rotary evaporator. The extract comprised of rosehip seed oil and an orange-red semi-solid. The rosehip seed oil was removed from the mixture by a siphon and was preserved for other purposes. The ethanolic semi-solid extract (ESE) was transferred to a pre-weighted, clean and oven-dried beaker. The procedure was repeated twice and the semi-solid extracts were combined, giving a bulk specimen of 18.2 g, from which smaller samples were weighed for the various investigations.

### 2.5 Evaluation of Antioxidant Activity and $IC_{50}$ Values

DPPH radical scavenging activity of the ESE of the press cake was performed according to the method described in literature [11, 12]. A stock solution of the semi-solid extract was prepared at a concentration of 3.0 mg of the extract per 1 mL of 50% methanol. Serial dilutions with the concentrations of 3000, 2000, 1500, 1000, 800, 500 and 200  $\mu$ g/mL were made from the stock solution. A negative control solution, i.e. a solution without the ESE was also included. A 0.1 mM DPPH solution, which served as oxidant, was prepared by dissolving 3.94 mg of DPPH in 100 mL methanol and was then stored in a dark cupboard to minimize chemical degradation. 100  $\mu$ L sample of the ESE solution was mixed with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris-HCl buffer maintained at pH 7.4. The mixed contents were shaken vigorously and then incubated in the dark cupboard for 30 minutes. The optical density of the incubated mixture was measured at 517.0nm using a spectrophotometer (MRS Spectro UV-11). Similarly, a stock solution of ascorbic acid, which served as positive control, was also prepared by dissolution of 3.0 mg of ascorbic acid in 1 mL of 50% methanol. Serial dilutions of concentrations of 3000, 2000, 1500, 1000, 800, 500, and 200  $\mu$ g/mL were also prepared accordingly. A solution without the

ascorbic acid (0 µg/mL) also served as the negative control and the optical densities were measured at 517.0 nm. The ability of the ethanolic extract and/or ascorbic acid to scavenge DPPH radical was calculated using the equation below:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_{\text{cont}} - A_{\text{test}})/A_{\text{cont}}}{1} \times 100$$

$A_{\text{cont}}$  = Absorbance of the negative control

$A_{\text{test}}$  = Absorbance in the presence of the extract or positive control [11].

The concentration of the extract that yields 50% inhibition is known as the  $IC_{50}$  value [13]. The  $IC_{50}$  values were estimated from plots of % Inhibition versus concentration.

## 2.6 Phytochemical Screening

Qualitative phytochemical screening of the ESE from the rosehip seed press cake was conducted using standard methods as described in literature [14-21]. The analysis targeted sixteen (16) phytochemical classes viz; flavonoids, alkaloids, tannins, terpenoids, saponins, anthocyanins, quinones, coumarins, sterols, glycosides, reducing sugars, proteins, amino acids, fatty acids, phenols and polyphenols. Different ESE samples were used to analyse for each of the classes.

The concentration of each phyto-constituent was estimated qualitatively through visual assessment of either precipitate deposition, colour transition and/or colour intensity; and the observations were denoted as: (+) - for low colour intensity and/or precipitate; (++) - for moderate colour intensity and/or precipitate; (+++) - for strong colour intensity and/or precipitate; and (-) – for no observable changes.

### 2.6.1 Detection of flavonoids

An amount of 0.50 g of ESE was dissolved in 2 ml of methanol and heated in a water bath. A small amount of magnesium powder was added to the mixture and few drops of concentrated hydrochloric acid were added. The formation of dark brown colouration which gradually turned deep red or pink colouration was taken as a positive test for the presence of flavonoids.

### 2.6.2 Detection of alkaloids

An amount of 5.0 mg of ESE was dissolved in 2 ml of distilled water and 3 drops of Wagner's

reagent (freshly prepared from 1.0 g iodine and 6.0 g potassium iodide dissolved in distilled water and diluted to 100 ml) were added to the mixture. The presence of alkaloids was indicated by the formation of a reddish-brown precipitate.

### 2.6.3 Detection of tannins

A 0.2 g sample of the ESE was dissolved in 2.0 ml of distilled water and heated in a water bath (95°C) for 5 min. The mixture was cooled and filtered. A few drops of concentrated sulphuric acid were added to the filtrate followed by addition of few (2-3) drops of 5% (w/v) ferric chloride solution. The presence of tannins was confirmed by the formation of blue black, green or blue-green precipitate.

### 2.6.4 Detection of terpenoids

A volume of 0.5 ml of distilled water was added to 0.25 g of ESE followed by addition of 1.0 ml of chloroform with vigorous shaking and swirling. The formation of reddish brown colouration on the interface upon the addition of 3.0 ml of concentrated sulphuric acid to the mixture indicated the presence of terpenoids.

### 2.6.5 Detection of saponins

A volume of 2.0 ml distilled water was added to 0.1 g of ESE and the contents were agitated in a test tube for 15 minutes. The presence of saponins was confirmed by the formation of a stable 1 cm layer of foam.

### 2.6.6 Detection of phenols and polyphenols

An amount of 0.5 g of ESE was dissolved in 1.0 ml of distilled water and was heated in a water bath (95°C) for 30 minutes. The resulting mixture was filtered and 3.0 ml of 5% (w/v) of ferric chloride was added to the filtrate, followed by the addition of 1.0 ml of 1% (w/v) potassium ferricyanide. The green colour was taken as evidence for the presence of simple phenols and the blue colour confirmed the presence of polyphenols.

### 2.6.7 Detection of anthocyanins

An amount of 2.0 g of ESE was dissolved in 2.0 ml of distilled water. About 1.0 ml of 2N sodium hydroxide was added to the extract solution and was heated for 5 minutes in a water bath. The presence of anthocyanins and betacyanins was confirmed by the formation of blue-green and blue colour respectively.

### 2.6.8 Detection of quinones

An amount of 0.25 g of ESE was dissolved in 0.5 ml of distilled water, followed by addition of 1.5 ml of concentrated hydrochloric acid. The deposition of green or white precipitate was taken as evidence for the presence of quinones.

### 2.6.9 Detection of coumarins

An amount of 0.3 g of ESE was dissolved in 1.0 ml of distilled water, followed by addition of 1.0 ml of 10% (w/v) sodium hydroxide and 1.0 ml of chloroform. The presence of coumarins was verified by the formation of yellow colour.

### 2.6.10 Detection of sterols

An amount 0.3 g of ESE was dissolved in 2.0 ml of chloroform then filtered off. 1.0 ml of concentrated sulphuric acid was added to the filtrate. The formation of two phases of which the chloroform phase turns red was indicative of sterols.

### 2.6.11 Detection of glycosides

An amount of 0.5 g of ESE was digested with hydrochloric acid and neutralized by sodium hydroxide. Two Fehling solutions; A and B, were prepared. Fehling solution A was prepared by dissolving 0.7 g of copper sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in 10.0 ml of distilled water containing 2 drops of concentrated sulphuric acid. Fehling solution B was made by dissolving 3.5 g of potassium tartrate and 1.0 g of sodium hydroxide in 10.0 ml of distilled water. Few drops of Fehling's solution A and B were added consecutively to the extract reaction mixture and the formation of red precipitate was taken as evidence for the presence of glycosides.

### 2.6.12 Detection of reducing sugars

An amount of 1.0 g of ESE was dissolved in 2.5 ml of distilled water. 2.0 ml of the extract solution was mixed with 2.0 ml of Benedict's solution. The mixed contents were heated in a boiling water bath for 5 minutes ( $95^\circ\text{C}$ ) until there was observable colour change. The formation of brick-red colouration confirmed the presence of reducing sugars.

### 2.6.13 Detection of proteins

An amount of 1.0 g of ESE was dissolved in 2.5 ml of distilled water. 2.0 ml of Biuret's reagent was added to 2.0 ml of the extract solution. The appearance of violet colour was taken as evidence for the presence of proteins.

### 2.6.14 Detection of amino acids

An amount of 1.0 g of ESE was dissolved in 2.5 ml of distilled water. To 2.0 ml of the extract solution, 2.0 ml of Ninhydrin reagent; prepared by dissolving 0.2 mg Ninhydrin in 10.0 ml acetone, was added and the mixture was incubated in a hot water bath for 20 minutes. The formation of purple colour was indicative of the presence of amino acids.

### 2.6.15 Detection of fatty acids

An amount of 1.0 g of ESE was dissolved in 2.5 ml of distilled water. 0.5 ml of the extract solution was mixed with 5.0 ml of diethyl ether. A drop of the mixture was added onto a filter paper and dried. The appearance of transparency on the filter paper confirmed the presence of fatty acids.

## 2.7 Determination of Total Phenolic Content

The total phenolic content (TPC) of the ESE from rosehip seed press cake was determined using the Folin-Ciocalteu assay [22] but with some modifications. A 1000  $\mu\text{g}/\text{ml}$  stock solution of gallic acid was prepared by dissolving 10.0 mg of gallic acid in 10.0 ml of 99% methanol. Two-fold serial dilutions of the 1000  $\mu\text{g}/\text{ml}$  stock solution to obtain a 15.6  $\mu\text{g}/\text{ml}$  working solution. Aliquots portions of 2.0 ml of the extract or gallic acid solutions were mixed separately with 1.0 ml of 1% (v/v) Folin-Ciocalteu reagent. The contents were allowed to stand for 4 minutes at room temperature followed by addition of 1.0 ml of 0.7M sodium carbonate solution. The mixtures were vortexed for 15 seconds and incubated for 25 minutes in the dark cupboard with occasional agitation. The optical density of the reaction mixture was measured at 765.0 nm using a spectrophotometer (MRS Spectro UV-11). The analyses were performed in triplicates.

## 2.8 Determination of Total Flavonoid Content

Total flavonoid content of the ESE was determined using the aluminium chloride calorimetric method described in literature [23]. A quercetin stock solution was prepared by dissolving 25.0 mg quercetin in 1.0 ml of 99% methanol. A standard calibration graph was obtained from various concentrations of the quercetin solution (25, 20, 15, 10 and 5  $\mu\text{g}/\text{ml}$ ). The concentration of the ESE was maintained at 1000  $\mu\text{g}/\text{ml}$ . 3.0 ml of extract or standard was added to a clean and dry test tube, followed by addition of 1.0 ml of 2% (w/v) aluminium chloride.

The mixed contents were incubated at room temperature for 15 minutes. The absorbance was taken at 420.0 nm using a spectrophotometer (MRS Spectro UV-11). The analyses were performed in triplicates.

### 3. RESULTS AND DISCUSSION

#### 3.1 Phytochemical Screening

The phytochemical analysis showed that the ethanolic semi-solid extract (ESE) from rosehip seed press cake contains flavonoids, alkaloids, terpenoids, phenols, polyphenols, sterols, glycosides, reducing sugars, proteins and fatty acids as shown in Table 1.

There is no comparable literature on the qualitative phytochemical profile of extracts from rosehip seed press cake. However, it has been previously reported that rosehip seeds contain phenolics, flavonoids, fatty acids, carbohydrates, sterols, amino acids, carotenoids and proteins. [7]. The study did not find free amino acids, but proteins were detected. The presence of more than 60% of the analysed phytochemicals in the moderate (++) to high (+++) concentration range (Table 1) indicated the potential of ESE in therapeutic and/or any other related applications.

#### 3.2 DPPH Radical Scavenging Activity

Table 2 summarizes the percentage inhibition of DPPH radical of the ESE from rosehip seed

press cake and that of ascorbic acid which was used as positive control. The ESE exhibited  $10.32 \pm 3.89$ ,  $25.75 \pm 2.65$ ,  $39.30 \pm 4.47$ ,  $48.14 \pm 4.22$ ,  $64.45 \pm 1.63$ ,  $72.89 \pm 3.01$  and  $76.06 \pm 3.48\%$  of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000  $\mu\text{g/ml}$ , respectively. On the other hand, ascorbic acid showed DPPH free radical scavenging activity of  $53.71 \pm 2.35$ ,  $54.07 \pm 0.72$ ,  $62.00 \pm 1.41$ ,  $64.75 \pm 2.27$ ,  $67.47 \pm 2.13$ ,  $77.04 \pm 0.44$  and  $79.27 \pm 1.07\%$  at the same concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000  $\mu\text{g/ml}$ , respectively. The results show that the ESE exhibited weak radical scavenging activity at low concentrations. However, its radical scavenging activity increased with concentration approaching that of the control, as indicated by the progressive decrease of the IR value, which approaches 1 (Table 3). Thus, ESE shows very promising radical scavenging activity at concentrations  $\geq 1500 \mu\text{g/ml}$  since its activity was very close to that of the positive control.

In the same manner, there was no comparable literature on DPPH radical scavenging activity of the pressed seeds extract. However, 80% methanol, 70% acetone and 60% ethanol extracts from normal rosehip seeds have been evaluated for their antioxidant activity using 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay in terms of trolox equivalents [7]. The methanol, acetone and ethanol extract were found to exhibit  $10.40 \pm 0.40$ ,  $10.71 \pm 0.10$  and  $7.29 \pm 0.80 \mu\text{mol TE/g}$  respectively [7].

**Table 1. Phytochemical analysis of the ethanolic semi-solid extract from press cake**

S/N	Phytoconstituents	Extract (ESE)
1	Flavonoids	+++
2	Alkaloids	+++
3	Terpenoids	+++
4	Tannins	-
5	Saponins	-
6	Phenols	+++
7	Polyphenols	+++
8	Anthocyanins	-
9	Quinones	-
10	Coumarins	-
11	Sterols	+++
12	Glycosides	+++
13	Reducing sugars	+++
14	Proteins	++
15	Amino acids	-
16	Fatty acids	+

(+) = Low concentration; (++) = Moderate Concentration; (+++) = High concentration; and (-) = Not detected.

**Table 2. DPPH radical inhibition by the ethanolic extract from rosehip seed press cake**

Substance	Concentration ( $\mu\text{g/ml}$ )						
	200	500	800	1000	1500	2000	3000
ESE	10.32 $\pm$ 3.89	25.75 $\pm$ 2.65	39.30 $\pm$ 4.47	48.14 $\pm$ 4.22	64.45 $\pm$ 1.63	72.89 $\pm$ 3.01	76.06 $\pm$ 3.48
Asc.acid	53.71 $\pm$ 2.35	54.07 $\pm$ 0.72	62.00 $\pm$ 1.41	64.75 $\pm$ 2.27	67.47 $\pm$ 2.13	77.04 $\pm$ 0.44	79.27 $\pm$ 1.07
IR	5.20	2.10	1.58	1.35	1.05	1.06	1.04

*ESE = Ethanolic extract, Asc. acid = Ascorbic acid. IR = inhibition ratio (= Asc. Acid % inhibition/EE % inhibition). All determinations were performed in triplicates (n=3) and the results were expressed as mean $\pm$ SD*

Table 3 summarizes the DPPH radical IC<sub>50</sub> value of the ESE. The ESE exhibited IC<sub>50</sub> value of 1367.06 µg/ml comparative to that of ascorbic acid being <200 µg/ml. The results showed that ESE is potent at concentrations >1000 µg/ml.

**Table 3. IC<sub>50</sub> value of the ethanolic extract**

S/N	Extract	IC <sub>50</sub> (µg/ml)
1	ESE	1367.06
2	Asc. acid	<200

*ESE = Ethanolic extract, Asc. acid = Ascorbic acid. (n=3) and the results were expressed as mean ± SD.*

As earlier stated, the activity of EE was much lower than that of ascorbic acid at lower concentrations while the activities converged at higher concentrations. As such, the IC<sub>50</sub> of EE was much higher than that of ascorbic acid by more than 6 times. Montazeri et al. (2011) also reported the IC<sub>50</sub> values for DPPH radical scavenging activity of methanol, water and hexane extracts from rosehip fruits to be 11.58, 15.14 and 224.6 µg/ml respectively [24]. The fruit extracts exhibited much higher antioxidant activity than the pressed seeds extract in this study [24]. The difference in antioxidant activity between the fruit and seed extracts suggests that the fruits are richer in antioxidants than the seeds.

### 3.3 Total phenolic and Flavonoid Content

The total phenolic content (TPC) of ESE was calculated from the regression equation of the Gallic acid calibration curve ( $y = 0.0009x + 0.3585$ ,  $R^2 = 0.9089$ ) and the result was expressed as mg of Gallic acid equivalents (GAE) per gram of sample in dry weight (DW) (Fig. 1). The total phenolic content of ESE was found to be 134.44 mg GAE/g DW.

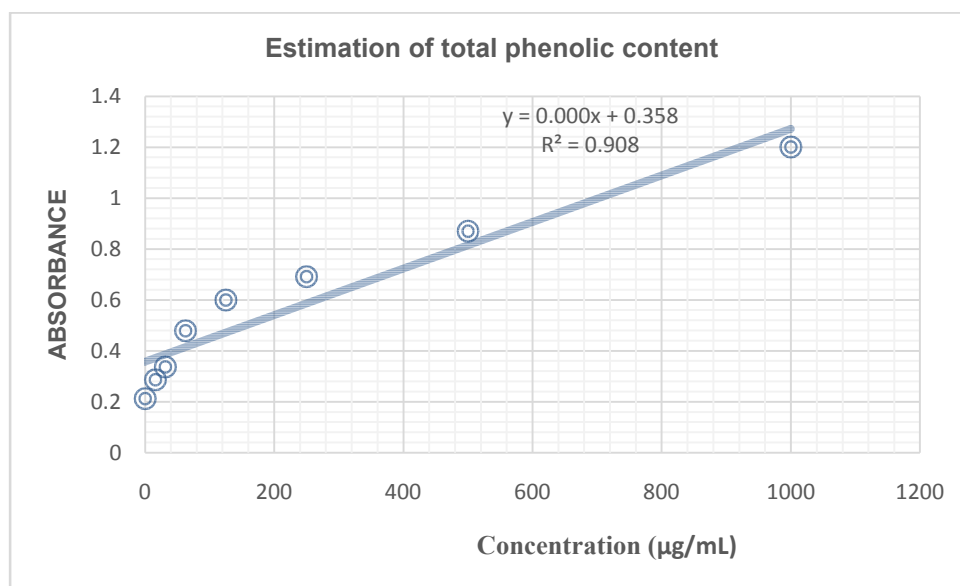
The total phenolic content of 80% methanolic extract of rosehip seeds was previously reported to be 2554 µg GAE/g seed [7]. In this study, total phenolic content of the pressed seeds was about 53 times higher than that reported by Ilyasoglu (2014) for untreated seeds [7]. This may be attributed to the fact that 99% ethanol has a much greater capacity to extract polyphenols and phenols as compared to 80% methanol. Elsewhere, the total phenolic content (TPC) of hexane, ethyl acetate, methanol, acetone, water and chloroform extracts from rosehips was found to be 63.76±2.6, 173.3±2.8, 424.6±1.8,

295.8±4.2, 220.2±5.9 and 145.8±3.3 mg GAE/g extract, respectively [24]. With the exception of hexane extract, the TPC of the ethanolic extract of the pressed rosehip seeds in this study was generally lower than that from the rosehip fruits, reported by [24]. This suggested that, the nature of plant materials and the solvents used are the major contributing factors in the measurement of TPC. Paunovic et al. (2019) also reported the TPC of the ethanolic extracts of fresh and dried rose hips in which 70% ethanol was used to effect extraction [25]. The TPC of the fresh and dried fruits was found to be 90.51±0.53 and 72.09±1.08 mg GAE/g DW, respectively [25]. The TPC recorded in this study for the pressed rosehip seeds was much higher than that of the rosehip fruits in Paunovic et al. (2019). The rosehip seeds constitute a larger portion of the rosehip fruits, hence the TPC of the pressed seeds in our study is greater. In addition, the discrepancy in TPCs suggests that 99% ethanol has a greater potential to extract polyphenols and phenols than 70% ethanol.

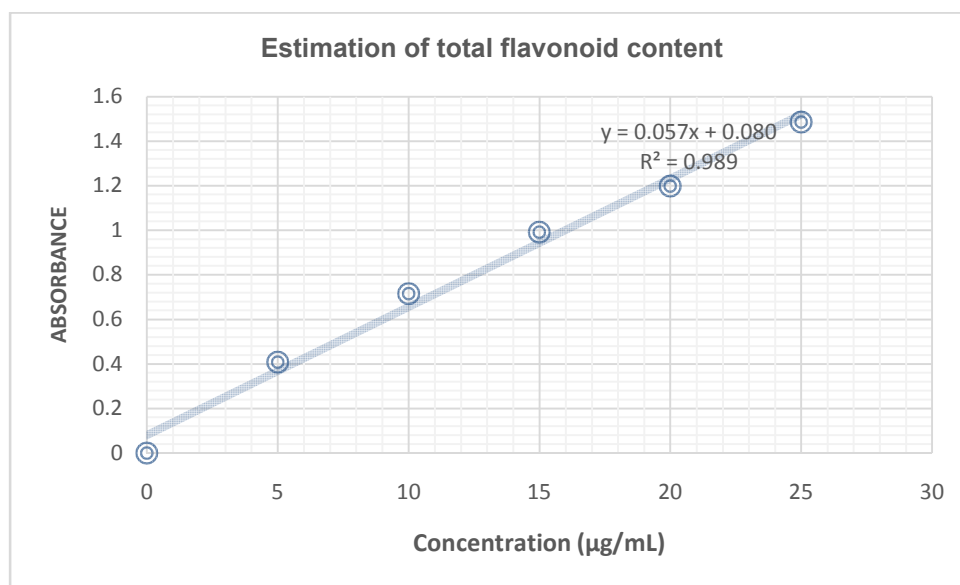
The total flavonoid content (TFC) of the sample was determined from the calibration plot ( $y = 0.0576x + 0.0801$ ,  $R^2 = 0.9891$ ) and was expressed as mg of quercetin equivalents (QE)/g of dried extract weight (DW) (Fig. 2). The total phenolic and flavonoid content of ESE were 134.44 mg GAE/g DW and 73.23 mg QE/g DW respectively.

In some studies, total flavonoid content (TFC) of hexane, ethyl acetate, methanol, acetone, water and chloroform extracts from rosehips was found to be 1.2±3.2, 8.1±2.8, 23.6±4.2, 18.4±2.2, 10.4±3.3 and 4.5±1.2 mg QE/g respectively [24]. The TFC of the ethanolic extract of the pressed rosehip seeds in this study was found to be higher than that of all the extracts from rosehips reported by [24]. Similarly, Paunovic et al. (2019) also reported the TFC of the ethanolic extracts of fresh and dried rosehips [25]. The TFC of the fresh and dried fruits was found to be 38.52±0.82 and 26.47±1.03 mg QE/g DW, respectively [25]. The TFC recorded in this study was much higher. In the case of the TPC, the nature of the solvent and plant part could be the contributing factors. Thus, the measured values of TPC and TFC serve as a useful guide on antioxidant content of the rosehips, but they do not represent the absolute content - they are bound to vary from one extraction to another.





**Fig. 1. Graph for estimation of total phenolic content**



**Fig. 2. Graph for estimation of total flavonoid content**

#### 4. CONCLUSION

The study evaluated the antioxidant activity, phytochemical screening, total phenolic and flavonoid content of the ethanolic semi-solid extract from rosehip seed press cake collected from The Rosehip Company, Lesotho. The phytochemical profile and the antioxidant activity of the ethanolic semi-solid extract from rosehip seed press cake indicate potential for utilization of the press cake pellets as a source of dietary antioxidant supplements. The ethanolic extract showed the presence of 62.5% of 16 screened phytochemical classes with most of them being

present in high concentrations. The total phenolic and flavonoids content of the extract was found to be 134.44 mg GAE/g DW and 73.23 mg QEF/g DW, respectively. The DPPH radical scavenging activity of the ethanolic extract was found to be between 10.32±3.89 and 76.06±3.48% at various concentrations. In addition, the IC<sub>50</sub> value of the extract was found to be 1367.06 µg/ml.

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## COMPETING INTERESTS

Authors have declared no conflicts of interest.

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