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DETERMINATION OF ANTIOXIDANTS AND ANTIBACTERIAL ACTIVITIES, TOTAL PHENOLIC, POLYPHENOL AND PIGMENT CONTENTS IN *NASTURTIUM OFFICINALE*

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Abstract

The anti-bacterial activity, total phenolic content, polyphenol, chlorophyll a, chlorophyll b, β-carotene and lycopene and the phenolic compound through HPLC were determined in methanolic extract of Nasturtium officinale. The antioxidant activity of the extract were also determined. Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis and Bacillus cereus, were screened by three methods, an agar well diffusion method, minimum inhibitory concentration and minimum bactericidal concentration. The zone of inhibition against Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis and Bacillus cereus were 1.92, 1.62, 1.82 and 2.56 cm respectively. The total phenolic contents and polyphenol were determined by Folin Ciocalteu reagent method and were found to be 4.5 and 5.12 mg GAE/g respectively. The chlorophyll a, chlorophyll b, β-carotene and lycopene were 0.235, 0.04178, 0.124 and 0.12654 (mg/100ml) respectively. The extract was subjected to HPLC analysis for antioxidants concentration. Morin and Chlorogenic acid (CGA) were detected at 12.858 and 6.079 retention times.

Keywords: *Nasturtium officinale*, High performance liquid chromatography, total phenolic contents, polyphenols, antioxidants, chlorophyll, pigments, inhibition.

Introduction

Medicinal plants have been used for the treatment of many human diseases over the century and have been very important in the health care delivery of every nation at one stage or the other (Dabanka et al. 2013). The development of bacterial resistance to presently available antibiotics has presented the need to search for new antibacterial agents (Jauda et al. 2013).

Different antioxidants are derived from different plants which provide a powerful defence to free radicals. Damage to the cell caused by free radicals is believed to play a central role in the aging process and in diseases progression. Antioxidants are our first line of defence to the damage caused by these free radicals and are critical for maintaining optimum health and well-being (Percival et al. 1998). Chlorophyll content is one of the important component of photosynthetic activity. It is of particular importance to precision agriculture and is an indicator of photosynthetic activity (Bojovic et al. 2015).

The family Cruciferae consists of 372 genera and about 4060 species which are dispersed and grown in running water and flooded places. Commercially, it is grown in unshaded pools of flowing clean water. Watercress falls in the genus Nasturtium of the Cruciferae family. The genus Nasturtium contains 7 species and it is perennial. It is not frost tender. The flowering time is from May to October and they ripe in July to October. The flowers are hermaphrodite and their pollination mainly occurs through bees, flies and self. The plant is self-fertile and has been noted for attracting wildlife (Stephan et al. 2015).

In the present study, the anti-bacterial activity, estimating total phenolic content, measuring polyphenol, finding pigments that include chlorophyll a, chlorophyll b, β-carotene and lycopene, the antioxidant activity and determining the phenolic compound through High performance liquid chromatography (HPLC) of *Nasturtium officinale* were performed.

Methods

Collection of plant materials

Nasturtium officinale plant was collected from local area Ouch Dir Lower. The plant samples were washed with clean water and kept in a shady place for drying at room temperature for 3 weeks. The dried plant then were powdered by grinding with the help of mechanical grinder. The powered plant was dissolved in methanol and kept it for one week with constant shaking at different intervals. After one week the mixtures were filtered through filter paper. The extract was obtained in semisolid form using rotary evaporator. It was then kept in open atmosphere to evaporate the remaining solvent and was thus converted into solid form. The crude extracts were kept in a refrigerator at a temperature of 4°C.

Antibacterial activity

Amoxicillin was used as a standard drug for the antimicrobial activity. Gram positive [Bacillus cereus (ATCC8035), Enterococcus faecalis (ATCC29212)] and Gram negative [Klebsiella pneumonia (ATCC13833), Escherichia coli (ATCC25922)] bacterial strains were used. Powders of the antibiotic amoxicillin (purity 100%) were accurately weighed and dissolved in sterile distilled water that give appropriate dilutions yield (0.03g/15ml) the required concentration. The standard solutions were stored at - 20°C. The growth media nutrient agar and the petri dishes were sterilized at 121°C for 3 hours in autoclave. Then four petri dishes were taken and the agar nutrient solution was transferred to each petri dish. Holes were made by cork borer in each agar plate at a proper distance from one another and standard antibiotic and crude extracts of watercress were introduced in the holes by using the micropipette. Now inoculate each agar plate with different bacterial strains with the help of cotton. After 24 hours of incubation period at 37°C in incubator the antimicrobial activities were determined from the diameter of the inhibition zone formed by the extracts of watercress around the holes. By measuring the zone diameter of inhibition created by watercress extract was compared with the inhibition zone created by standard antibiotic (amoxicillin). The Minimum Inhibitory Concentration was found by preparing five different solution of watercress extracts 0.02, 0.04, 0.06, 0.08 and 0.1 (making 10 ml of such solution). Now nutrient broth solution was prepared and 9 ml of broth solution was taken in five test-tube. After that add 1, 1 ml from each of the five extract solution to the test-tube and one type of bacterial strain to these five test tube were added. The test tube were placed in incubator for 24 hours and after this minimum inhibitory concentration against selected bacteria were found. After MIC, MBC was carried out, after 24 hours MIC was found and after 3 days MBC was determined. From changes in the turbidity in the test tube was determined.

Determination of free radical scavenging activity

First of all 0.039gm of DPPH was accurately weighted with the help of digital balance and dissolve in the

distilled methanol to give an appropriate solution of 100ml (0.039gm/100ml) of the required concentration.

1 gm of extract was accurately weighted and dissolved separately in the distilled methanol to give the required solutions of 20 ml to get the required concentrations. Then this solution was stored as *Nasturtium officinale* stock solutions. One solution of 5ml pure distilled methanol + 0ml *Nasturtium officinale* solution. This solution was used as control solution. After these five dilute solutions were used as control solutions were prepared from Nasturtium officinale.

Then 1 ml of stock solution of DPPH was added to the control solution and diluted solution of extract solution. Then all these solutions were kept in dark place for 30 minutes. After this their absorbance reading was taken at 517 nm of maximum wavelength with the help of spectrophotometer in here by the given formula;

$$\%RSA = \frac{\text{Control solution absorbance} - \textit{Nasturtium officinale} \text{ solution absorbance}}{\text{Control solution absorbance}} X100 \quad (1)$$

Total phenolic acid content

The total phenolic of *Nasturtium officinale* was estimated by Folin Ciocalteu reagent method using Gallic acid as standard phenolic compound. 1g of plant extract was added into a flask containing 9 ml of distilled water. Then 1 ml of Folin Ciocalteu reagent was added and the mixture was mixed thoroughly. After 5 minute of incubations, 10ml of 7% Na2CO3 were added. Then the mixture was diluted to 25 ml with the addition of 4 ml of distilled water. Then the mixture was incubated at room temperature for 90 minute. Finally the absorbance was measured using UV/Visible spectrophotometer at 750 nm. The total phenolic acid content was expressed as mg GAE/g sample.

Total Polyphenol Assay

Total polyphenol was measured using Folin Ciocalteu method. The 1 g extract were mixed with 2 ml of Folin Cioccalteu reagent and 20 ml of H2O, and incubated at room temperature for 3 minute. Then added 10 ml of 20 % of Na2CO3 to the mixture. Then total polyphenol was determined after 1 hour of incubation at room temperature .The absorbance of the resulting mixture was measured at 765 nm .Quantification was done with respect to the standard curve of Gallic acid. The result was expressed as GAE, mg /g of dry weight.

Determination of Pigments Contents

Pigments such as Chlorophyll α, Chlorophyll b, β-carotene and Lycopene were determined by Spectrophotometer. 1 gm of each sample was mixed with acetone-hexane (4:6) and then placed on shaker for 1 hour. After shaking, the absorbance were noted at 453, 505, 645 and 663 nm respectively and the results were expressed as mg/100 ml.

Determination of phenolic contents through HPLC

For the preparation of extracts, 10 mL of methanol and water were mixed with 1 gram from the powdered sample. These mixtures were then heated on hot plat on 70 0C for 1 hour. After cooling, the samples were filter with Whatman filter paper then these filter samples were centrifuged at 6000 rpm for 15 minutes, after centrifugation it is filter through syringe filtration or micro filtration into HPLC vials. The vials containing the samples were labelled with proper code. The determination of phenolic compounds were carried out by means of Agilent 1260 (HPLC) system. The separation was achieved via Agilent Zorbax Eclipse C18 column. The identification was performed by using retention times, available standards and UV spectra. Quantification of the identified compounds was on the basis of percent peaks values. HPLC chromatogram of *Nasturtium officinale* is shown in the figure 2.2. Only polyphenolic compound was identified. The detailed identification of compound with his peaks position in chromatogram and retention time (RT) is given in figures 2.1. Morin was eluted at retention time 12.858 min and was identified from the standard Morin peaks in the standard chromatogram. The injection volume was 10 micro litres and lambda max was 320 nm. Chlorogenic acid (CGA) was eluted at retention time 6.079 min and was identified from the standard CGA peaks in the standard chromatogram. The injection volume was 10 micro litres and lambda max was 320 nm.

Results

Antimicrobial activities of the methanolic extracts of *Nasturtium officinale* (watercress)

The methanolic extract of *Nasturtium officinale* was tested for its antimicrobial activity and their values are shown in the graphical representation figure 1.
The MIC and MBC of watercress against *Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis* and *Bacillus cereus* are given below in table 1.
As new drug-resistant bacterial strains emerges, herbal drugs are being looked as very important source for

discovery of new agents for treating various ailments related to bacterial infections. We conducted a prospective observational study of antibacterial activity of methanolic extract of watercress. The maximum antibacterial activity observed by amoxicillin against *E.coli* was 4 cm. This standard showed significant zone of inhibition against all bacterial strains used as compared to the extracts. The present study correlates with the study of (Hamayun *et al.* 2015) in which he studied the antibacterial activity of watercress extracts against Escherichia coli, Salmonella typhi, Streptococcus pneumoniae and Proteus vulgaris by agar well diffusion method.

Antioxidant activity

The free radical scavenging activities of the methanolic extract of *Nasturtium officinale* are shown in table 2.

Oxidative stress causes serious cell damage leading to a variety of human diseases therefore we designed this study to investigate the possible antioxidant potential of methanolic extracts of watercress. It was found that the free radical-scavenging activities of the extracts increased with increasing concentration which is clear from the figure. The present study correlates with the study of (Ozen *et al.* 2015) in which he investigate the antioxidant activity of the watercress extracts both in vivo and in vitro. The antioxidant capability of watercress leaves was evaluated using the Extracts were evaluated for total antioxidant activity by ferric thiocyanate method. The present study also correlates with the antioxidants activities obtained by total antioxidant capacity method (Mazandarani *et al.* 2013) in which they studied the secondary metabolites content and antioxidant activity in aerial parts of *Nasturtium officinale* R. Br., at various altitudes.

Total phenolic acid content

The total phenolic of *Nasturtium officinale* was estimated by Folin Ciocalteu reagent method using Gallic acid as standard phenolic compound. The total phenolic acid content was expressed as mg GAE/g sample and were 5.25 that were found by formula as given below

$$TPC = \frac{Ccal * D * V(ml)}{wt} \qquad (2)$$

The total phenolic acid content was determined by Folin Ciocalteu reagent method (Andressa *et al.* 2013), the absorbance was measured using spectrophotometer at 750 nm .The total phenolic acid content was expressed as mg GAE/g sample values. This study correlates with the total phenolic acid contents determined in (Mazandarani *et al.* 2013), using calorimeter method.

Total Polyphenols

Total polyphenol was measured using Folin Ciocalteu method. Quantification was done with respect to the standard curve of Gallic acid. The result was expressed as GAE, mg /g of dry weight is 5.12 as found by formula 1.

The total polyphenol were measured using Folin Ciocalteu method, the absorbance of the resulting mixture was measured at 765 nm .Quantification was done with respect to the standard curve of Gallic acid. The result was expressed as GAE, mg /g of dry weight. This study correlates with (Silverira *et al.* 2014), in which the total polyphenol contents were determined for the extract obtained after homogenizing 1 g of frozen watercress leaves with 3 mL of methanol/water solution.

Determination of Pigments Contents

The pigment contents were determined by the simple method devised by Nagata and Yamashita (Nagata *et al.* 2009). The equation devised by them is given as follow:

Chlorophyll a (mg/100ml) = $0.999A_{663}$-$0.0989A_{645}$

Chlorophyll b (mg/100ml) = $-0.328A_{663}$+$1.77A_{645}$

Lycopene (mg/100ml) = $-0.0458A_{663}$+$0.204A_{645}$+$0.372A_{505}$-$0.0806A_{453}$

β- Carotene (mg/100ml) = $0.216A_{663}$-$1.22A_{645}$-$0.304A_{505}$+$0.452A_{453}$

In this we determined different pigments such as Chlorophyll α, Chlorophyll b, β-carotene and Lycopene with the help of spectrophotometer. These pigments showed absorbance at 453,505,645 and 663. This study correlates with (Gonclaves *et al* 2009), in which he determined different pigments at three different temperatures.

Determination of phenolic contents through HPLC

The two compounds that were identified, While comparing with the standard curve of the phenolic compounds were Morin and CGA, these compound were eluted from the column with retention time 12.858 and 6.079, using single point calibration formula the concentration of these compound were calculated and were found to be 99011.78186 µ/g and 9.271032334 µ/g respectively, the formula as

$$Cx = \frac{Ax * Cs\left(\mu g/_{ml}\right) * V(ml)}{As * Sample\ (wt\ in\ g)} \qquad (3)$$

Where

C_X = Concentration of unknown

A_S = Peak area of standard

A_x = Peak area of unknown

Cs = Concentration of standard

The HPLC chromatogram of *Nasturtium officinale* showed two Polyphenolic compounds that were identified through comparing with standard chromatogram containing eight standard antioxidant. These compounds were shown with their respective peak position, retention time, and wavelength and identification references. Of these compounds one was identified as Morin which eluted at width minute 0.1705 and identified from the standard.

At the retention time of 6.079 min, CGA was eluted having area 22.18590. This study correlates with (Boligon *et al* 2013), in which he study the phenolic contents through HPLC.

Discussion

In this study, the antibacterial and antioxidant activity of methanolic extracts of *Nasturtium officinale* were determined. The extract show antibacterial activity, the highest antibacterial activity was recorded for Bacillus cereus. The MIC and MBC were also determined, the MIC against E.coli was 0.06, against Klebsiella pneumonia was 0.04, against Enterococcus faecalis was 0.08 and Bacillus cereus was 0.06. The MBC was also determined against E.coli was 0.10, against Klebsiella pneumonia was 0.08, against Enterococcus faecalis was 0.10 and against Bacillus cereus was 0.08. The extract also showed high free radical scavenging activity and with increase concentration there was an increased antioxidant activity. The total phenolic acid content also determined and were found to be 5.25 mg /g. the total polyphenol were also determined and were found to be 5.12 mg /g. the pigment contents chlorophyll a, chlorophyll b, β-carotene and lycopene were determined and there their Concentration (mg/100ml) are 0.235, 0.04178, 0.124 and 0.12654 respectively. The presence of Morin and CGA were confirmed through HPLC and their concentration was 99011.78186 µ/g and 9.271032334 µ/g respectively.

The *Nasturtium officinale* extracts in the form of methanolic extracts exhibit antibacterial, antioxidants and other phytochemicals and as a potent candidate is used as medicinal plant. The present study will open a new research area to be investigated in future.

References

Andressa B, Lopes GC, De Mello JCP. 2013. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from Limonium Brasiliense L. Molecules. 18:6852-6865

Bojovic B, Stojanovic J. 2005. Chlorophyll and carotenoid content in wheat cultivars as a function of mineral nutration. Arch. Biol. Sci., Belgrade. 57:283-290.

Boligon AA, Janovik V, Pivetta CR, Pereira, Da Rocha JBT, Athayde ML. 2013. HPLC Analysis of Polyphenolic Compounds and Antioxidant Activity in Nasturtium officinale. INT J FOOD PROP. 16:61-69

Dabanka CP. 2013. Antibacterial activity of Phyllantus amarus (schum and thonn) extract against salmonella typhi causative agent of typhoid fever. The department of environmental science, Kwame Nkrumah university of science and technology.

Gonclaves EM, Cruz RMS, Abreu M, Brandao TRS, Silva CLM. 2009 Biochemical and colour changes of watercress (Naturtium officinale R.Br) during freezing and frozen storage" J Food Eng. 93:32–39

Hamayun K, .Jan SA, Mehwish, Rabia S, Zahid K, Aftab A, Safi SZ, Imran M. 2015. Nutritional Composition, Antioxidant and Antimicrobial Activities of Selected Wild Edible Plants. J. Food Biochem. 40:61-70

Jouda MM. 2013. The Antibacterial Effect of Some Medicinal Plant Extracts and their Synergistic Effect with Antibiotic and Non-antibiotic Drugs. Islamic University-Gaza.

Mazandarani M, Momeji A, Moghaddam PZ. 2013. Evaluation of phytochemical and antioxidant activities from different parts of *Nasturtium officinale* R.Br. in Mazandaran. IJPP. 3:659-664

Nagata M, Yamashita I. 2009. Simple method for simultaneous determination of chlorophyll and carotenoids in Tomato fruit. J JPN SOC FOOD SCI 10:925-928.

Ozen T. 2009. Investigation of antioxidant properties of *Nasturtium officinale* (Watercress) Leaf extracts. Acta Poloniae Pharmaceutica and Drug Research. 66:187-193.

Percival M. 1998. Antioxidants. NUTR. Rev. 31:1-4.

Silverira A.C, Araneda C, Hinojosa A, Escalona VH. 2014. Effect of non-conventional modified atmosphere packaging on fresh cut watercress (*Nasturtium officinale* R. Br.). Postharvest Biol. Technol. 92:114–120

Stephan JM. 2015. Watercress- *Nasturtium officinale* R.Br. University of Florida, US State.

Table 1: MIC and MBC activity of *Nasturtium officinale* against different bacteria

Bacteria	*Escherichia coli*	*Klebsiella pneumonia*	*Enterococcus faecalis*	*Bacillus cereus*
MIC	0.06	0.04	0.08	0.06
MBC	0.10	0.08	0.10	0.08

Table 2: Antioxidant activity

Concentration	20 ppm	40 ppm	60 ppm	80 ppm
% RSA	32.97	42.64	57	87.19

Table 3: Chlorophyll absorbance on different UV light ranges

Pigments	**Concentration (mg/100ml)**
Chlorophyll A	0.235
Chlorophyll B	0.04178
β-carotene	0.124
Lycopene	0.12654

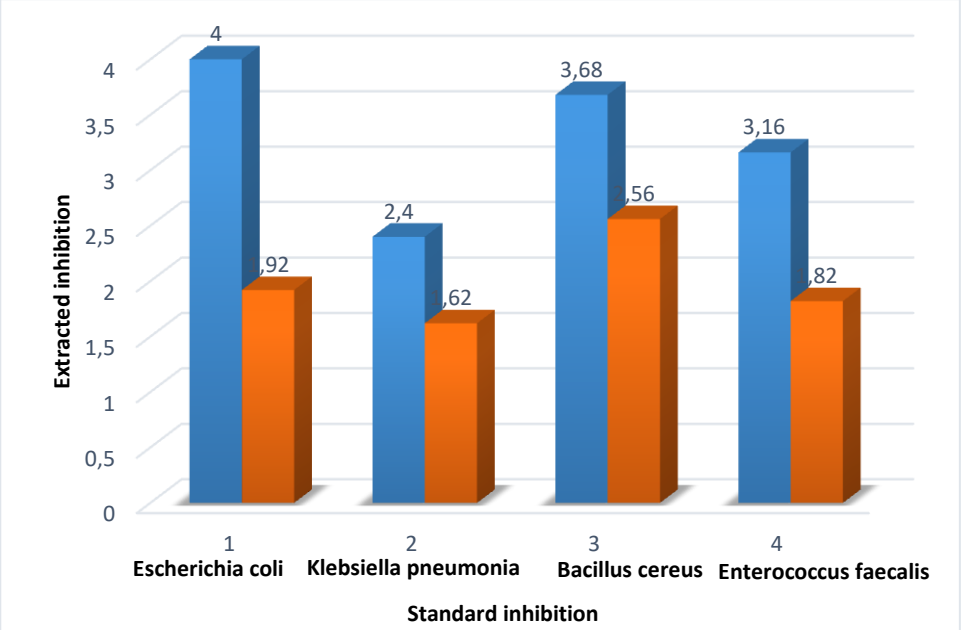

Figure 1: Graphical representation of Antibacterial activity of methanolic extracts of *Nasturtium officinale.*

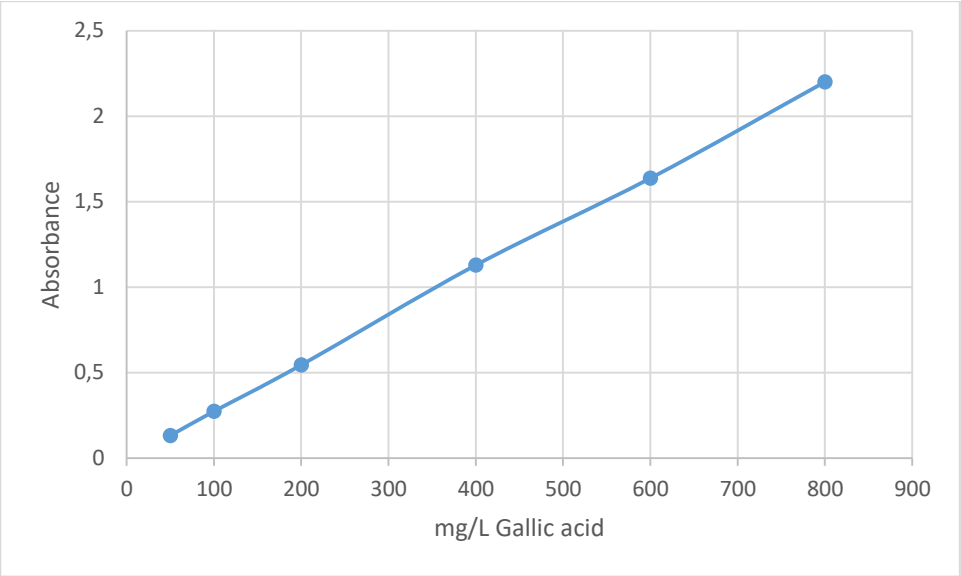

Figure 2: Folin Ciocalteau Gallic Acid Standard Curve

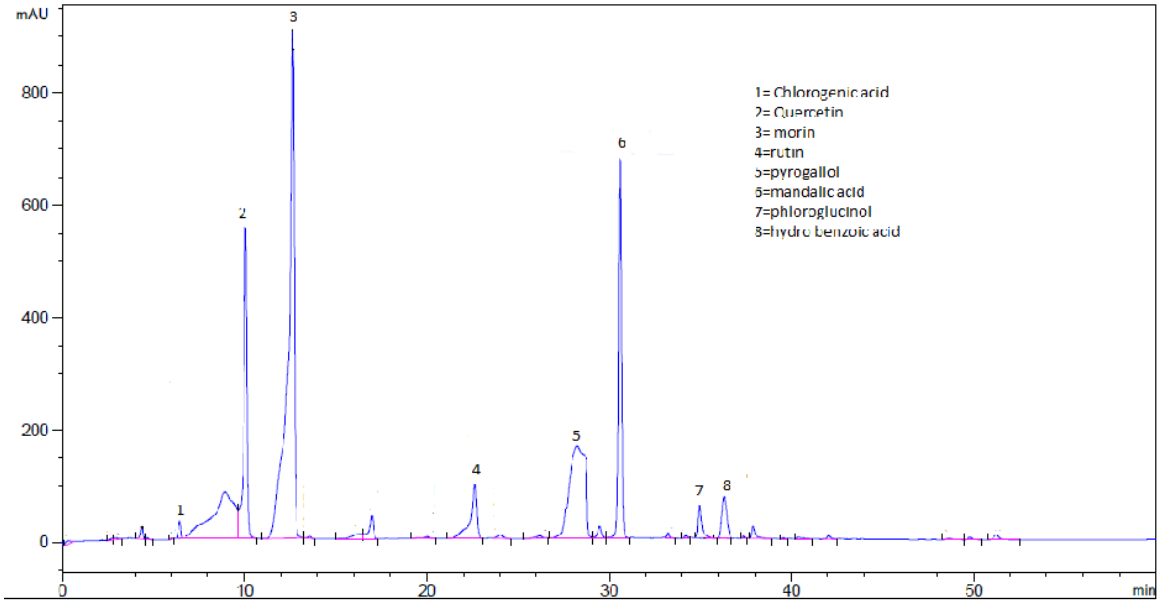

Figure 3: A representative HPLC-UV chromatogram of the standard phenolic compounds.

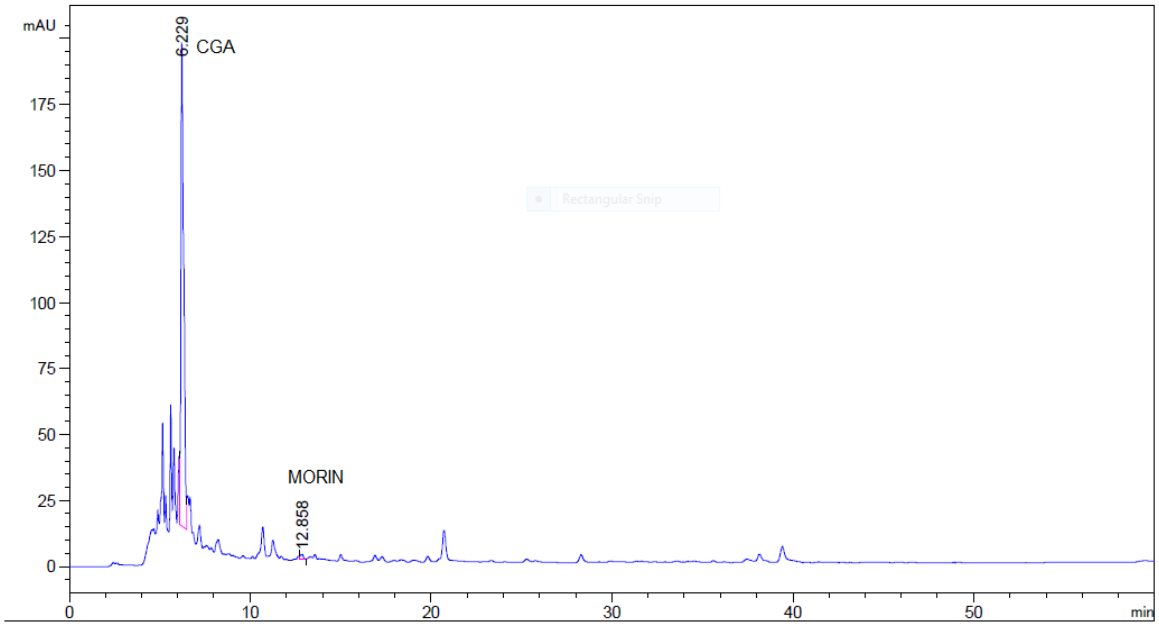

Figure 4: A representative chromatograms of phenolic compound in *Nasturtium Officinale,* Morin and Chlorogenic acid.