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High-performance liquid chromatographic analysis of the extracts of ginger, hibiscus and colves herbal plant

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Abstract
A high performance liquid chromatographic method was carred out to estimte niacine, ascorbic acid and gallic acid in ginger, hibiscus and colves samples using aqueous extract; a very simple method was performed. A C18 column was used with a gradient elution of mobile phase at a flow rate of 0.38 cm^3/min. The results obtained showed the presence of niacine and gallic acid, in all herbal samples. Ginger gave high concentration of niacine (11.48 ppm), then hibiscus (1.04 ppm) and colves (0.525ppm). Ginger gave a high concentration of gallic acid (56 ppm), then hibiscus (12.05 ppm) and colves (2. 265 ppm). However, The concentration of vitamin C was not detected in the three herbal samples because extraction method used might not be suitable for its extraction.

Keywords: HPLC /MS, ascorbic acid, gallic acid, niacin, aqueous extract

1. Introduction
Aqueous extract of ginger , hibiscus and colves plants, is rich in many chemical compounds, for example ascorbic acid, niacin and gallic acid. Vitamins A, C and E obtained from vegetables and fruits protect cells and tissues against damaging effect of free radicals (Mukherjee2009). On the other hand, some ingredients from natural products are incorporated in cosmetic preparations owing to their various therapeutic properties, e.g. antiaging, moisturizing, antioxidant, antiinflammatory and antimicrobial effects, hair stimulants, etc. (Aburjai2003) [4]. Hibiscus is a typical plant of tropical climates found in the regions of mangroves in significant quantities. Hibiscus plant have pharmaceutical and cosmetics benefits. Previous pharmacological investigations of the genus hibiscus plants indicated the presence of species with useful biological activities. The studies conducted to date have demonstrated that plants of the Hibiscus containing anthocyanins that use for hair dyeing (Rosa RM 2006) [6]. Ginger and clover are the medicinal plants known for their therapeutic benefits since ancient times, and their use in several forms either fresh or dried , powder , or oil. (Shady 2013, Widyarini 2001) [2, 5]. The main function of niacin is to help to maintain the structure of the blood cells and improves blood circulation (Eshe 2017) [3] Gallic acid is commonly used in the pharmaceutical industry (Fiuza2004).
Owing to the remarkable antioxidants properties of ascorbic acid, it is widely employed in pharmaceutical and cosmetic industry (Tapan 2016).

2. Materials and Methods
Chemicals and reagents
- Standards chemicals like niacine , ascorbic acid and gallic acid.
- The HPLC-grade solvents such as, methanol, acetic acid from Merck (Germany).

Instrumentation
High- performance liquid chromatography (HPLC) equipped with an analytical column A C-18 LUNA (5 micron 25 cm×4.6 mm). prominence UFLC Shimadzu Corporation.

Methods
Preparation of aqueous extract
About 5 g of samples powder was weighed accurately, transferred to falcon tube, extracted with 30% of methanol and 70% distilled water and acidified to pH 3 with phosphoric acid, by shaking for 30 min .The samples then filtered with 0.45 µL, and subjected to determination of niacine, ascorbic acid and gallic acid in ginger , hibiscus and colves .

Preparation of mobile phase
The mobile phase was prepared by mixing methanol and formic acid (0.1% in water) for gradient elution system. Table 1 shows the chromatograph operating conditions.

Table 1: HPLC instrument Operation conditions

Time min	Methanol(B)	formic acid 0.1% in water(A)
0.01	5	95
5	90	10
9	5	95
10		

Flow rate : 0.38 cm^3/min
Column Temp : 40° C
The injection volume : 10µL

Mass spectroscopy (MS) Conditions
Instrument :MS 2020(Shimadzu corporation).
Ionization :ESI positive (DUIS PROBE)
Dl :400
Dry gas :5 L

Results and discussion

Determination of niacine, vitamin C and gallic acid in ginger, hibiscus and colves using high-performance liquid chromatography/mass spectroscopy (HPLC/MS)

Column chemistry, solvent type, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized to eliminate interference from solvent and other compounds. For HPLC analysis, initially various mobile phases were tried in attempts to obtain the best separation and resolution between Niacine, vitamin C and gallic acid. The mobile phase consisting of gradient elution of methanol and formic acid (0.1% in HPLC-grade water) was found to be an appropriate mobile phase allowing adequate separation using C-18 column at a flow rate of 0.38 cm^3/min. Niacine, vitamin C and gallic acid were chromatographically determined in aqueous extract samples of ginger, hibiscus and colves .The calibration curves of niacin, vitamin C and gallic acid standards are shown in figures 1, 2 and 3, respectively.

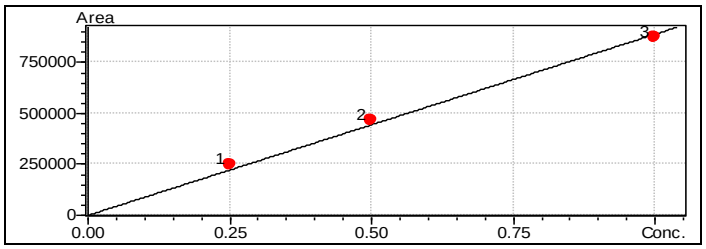

Fig 1: Calibration curve of niacin standards

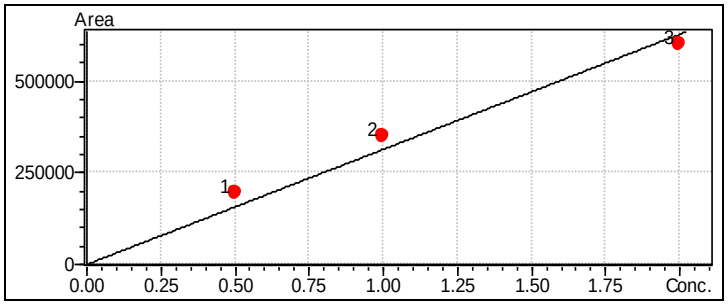

Fig 2: Calibration curve of gallic acid standards

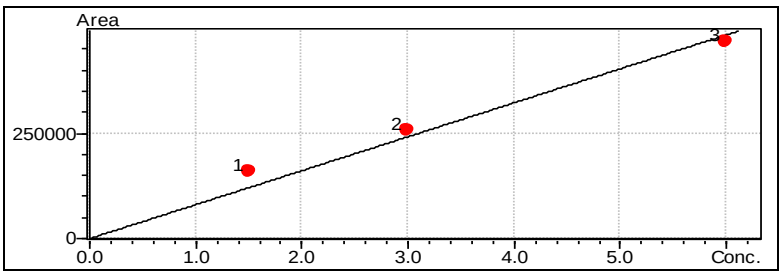

Fig 3: Calibration curve of vitamin C standards

Figure 4, 5 and 6, shows the HPLC chromatogram of hibiscus, ginger and colves of level 1, level 2 and level 3 standards, respectively.

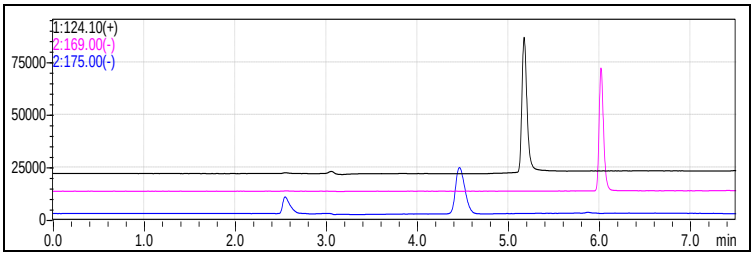

Fig 4: The HPLC chromatogram of hibiscus, ginger and colves of level 1 standards

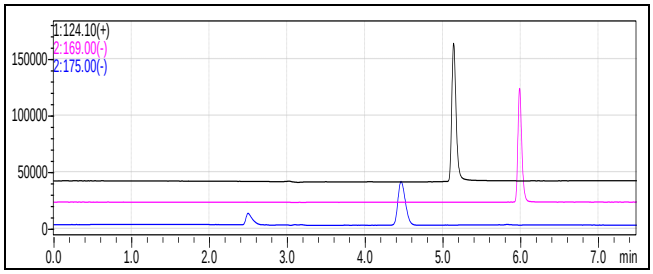

Fig 5: The HPLC chromatogram of hibiscus, ginger and colves of level 2 standards

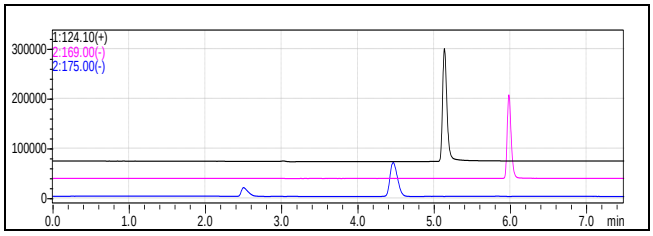

Fig 6 : The HPLC chromatogram of hibiscus, ginger and colves of level3 standards

HPLC chromatogram of ginger, colves and hibiscus are shown in figure 7, 8 and 9 respectively.

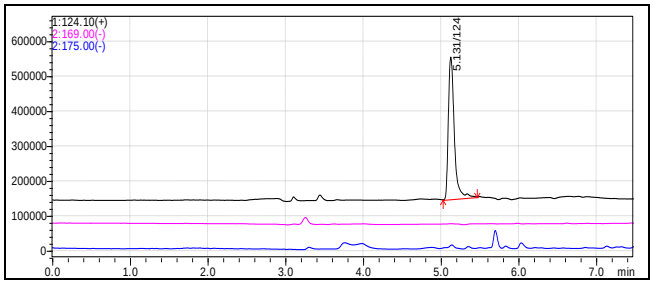

Fig 7: The HPLC chromatogram of ginger

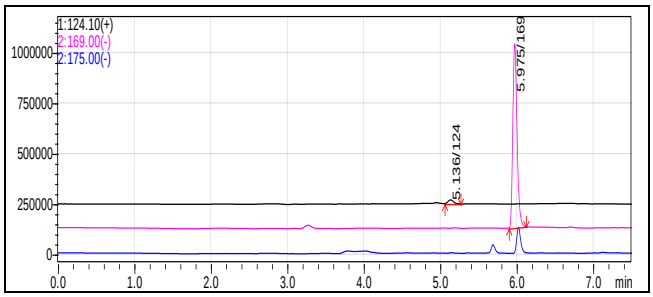

Fig 8: The HPLC chromatogram of colves

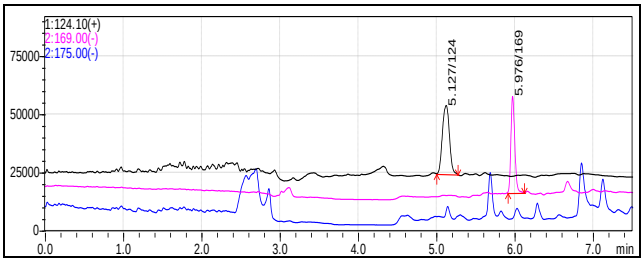

Fig 9: The HPLC chromatogram of hibiscus

Niacine, vitamin C and gallic acid in ginger, colves and hibiscus were extracted with 30% of methanol and 70% distilled water, acidified to pH 3 with phosphoric acid, and determined using high-performance liquid chromatography/ mass spectroscopy (HPLC/MS). The results obtained are shown in Table 2.

Table 2: Results of (HPLC/MS) determination of niacine, vitamin C and gallic acid in ginger, hibiscus and colves

Samples	Conc. Of Niacine ppm	Conc. of Gallic acid ppm	Conc. of vitamin C ppm
Hibiscus	1.04	12.05	*N.D.
Colves	0.525	2. 265	N.D.
Ginger	11.48	56	N.D.

*N.D.: not detected

The results obtained showed the presence of niacine and gallic acid, and the absence of vitamin C, in all herbal samples. Ginger gave high concentration of niacine (11.48 ppm), then hibiscus (1.04 ppm) and Colves (0.525ppm). Ginger gave a high concentration of gallic acid(56 ppm), then hibiscus (12.05 ppm) and colves (2. 265 ppm). However, The concentration of vitamin C was not detected in the three herbal samples because extraction method might not suitable for its extraction.

Conclusion

The applied HPLC method represented an excellent technique for simultaneous determination of niacine and gallic acid in aqueous extract of ginger, hibiscus and colves plants. The method gave a good resolution among niacine and gallic acid with gradient elution. The concentration of vitamin C was not detected in the three herbal samples because the extraction method used might not be suitable for its extraction.

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