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COMPOSITION, BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF THE AQUEOUS AND ETHANOLIC EXTRACT FROM SAUSSUREA LAPPA ROOTS

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Abstract

Plant extracts have been widely used for their anti-inflammatory, antifungal, antiviral and antibacterial properties. The present study was conducted to investigate the potential use of S. lappa roots as a source of bioactive compounds. Boiled aqueous and ethanol solvent fractionates were evaluated for antioxidant efficacy. The chemical composition, antioxidant activity, phenolic and flavonoids compound properties, total carotenoids and vitamin C of the extracts was examined using various in vitro assays. The total contents of phenols and flavonoids were determined by the Folin-Ciocalteu's Colorimetric and the aluminum nitrate method, respectively. Phenols and flavonoid compounds were detected and identified in the active fraction based on spectrophotometer and HPLC analysis data. The results showed that protein content was 2.74and 4.37%, while fat content was 5.74and9.57% for the aqueous and ethanol extracts, respectively. Higher content of vitamin C and carotenoids was observed for ethanolic extract, reaching 35.06 and 4.07 (mg/100g), compared to the content in the aqueous extract of 24.53 and 0.96 (mg/100g), respectively. The extractsofS. lappa roots were appreciably different for the studied solvents as ethanol extract was more active than aqueous extract. The higher antioxidant activity of ethanol extract appeared be related to the high amount of phenols and flavonoid compounds and higher number of phytochemicals. In the aqueous extract, the highest flavonoids detected included rutin, myricetin, quercitin and neringein and the largest amounts of phenols were Vanillic acid and Salicylic acid. In the ethanolic extract of S.lappa root, the most abundant compounds were Rutin and Myricetin, Salicylic acid, Vanillic acid, Caffeine and Pyrogallol). In conclusion, S. lappa roots used to help prevent antioxidant stress as a value-added product, and natural food supplement.

Keywords: Saussurealappa, Costus root, Phytochemicals, Antioxidant activity, Phenols and Flavonoids compounds, Carotenoids.

Introduction

Over the past decades, there has been a growing interest in investigating natural products from various sources, particularly from higher plants to discover new antimicrobial and antioxidant agents, which have been shown to have in vitro antimicrobial and beneficial properties (Lohitha, et al., 2010 and Mothana, et al., 2010).

Medicinal plants have beneficial nature for human health. Developments in the field of modern medicine temporarily weakened traditional herbal medicine. It has been reorganized and worldwide “herbal renaissance” flourished (Sumathi,& Parvathi, 2010). In developing countries, approximately 60-80% of the world’s population has been relying on traditional medicines to treat common diseases to combat serious diseases (Tekeli,et al., 2011).

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The root of Saussurealappa (Asteraceae), known as Costus has been used in the traditional medicine of many countries in the treatment of many diseases and ailments (Butola and Samant, 2010). Saussurealappa has active molecules (Hegde, et al., 2014) that have medicinal effects as immune modulation, antidiabetic, anti-inflammatory, antimicrobial, hypolipidemic, and antiparasitic (Shanmugam, 2010 and Wang,et al., 2010). Thus, it has been utilized as a part of the dental pathology for being viable against Streptococcus mutans that induce dental caries (Remya and Daniel 2012).

Costus (Saussurealappa) is an aromatic perennial plant that is widely utilized in various indigenous medical systems globally for treating a variety of disorders, e.g., diarrhea, tenesmus, dyspepsia, vomiting, and inflammation (Toussonet al., 2019; Toussonet al., 2020). Costus is rich in antioxidants and has anti-hepatotoxic, antifungal, antihelmintic, antidiabetic, anti-inflammatory, anti-tumor, anti-ulcer, antimicrobial, and immunostimulant effects (Nadda et al., 2020).

Moreover, Semwal, et al., (2020) reported that S. costus(S. lappa) was evaluated for different pharmacological activities. The root, which is used in traditional medicine, showed activity against cancer, microbial infections, inflammations, ulcers, etc. It proved that Saussureaccostushas therapeutic importance as anti-gastric, anti-asthmatic, antispasmodic, antimicrobial, and anti-inflammatory.

The ethanol extract of S. lappa (synonymous S. costus) demonstrateda wide spectrum of antimicrobial activity against some human pathogens (Hassonet al., 2013). Further, other bioactive properties of S. costusroots, e.g., anti-ulcer, anti-inflammatory, hepatoprotective, immunomodulator, hypoglycaemic, spasmolytic, anticonvulsant, antidiarrheal, and antiviral activity were noted (Al Ghashamet al., 2017). The ethanolic concentrate of the essential bases in Saussurealappa has antioxidant activity factor action suppressing nitric oxide generation in lipopolysaccharides. Isolated molecules and their mixture showed antimicrobial effects (Basudan, 2018).

Natural products are rich in bioactive compounds so, it is necessary to develop a new technology in discovering new drugs based on them (Mohamed,et al., 2018). However, poor aqueous solubility, insufficient permeability, and suboptimal bioavailability limit their uses. Therefore, several approaches have been developed to improve the solubility, permeability, and bioavailability of natural products (Yusif,et al., 2016).

As a new source of antimicrobial agents, many research groups involved in the field of medicinal plants have paid great attention to these natural resources (Hassonet al., 2013). Herbal plants have been widely used due to their numerous health benefits and are safe and less expensive than conventional drugs. They are known to treat some diseases (Magtulis&Itable 2020).

The present study aimed to determine the nutritional value and profile of the bioactive compounds of two solvent extracts from the roots of costus and to evaluate their antioxidant activity.

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Materials and Methods

Plant material

The roots of S. Costus were purchased from the local market in Giza, Egypt. Roots were washed with clean water and air-dried. Dried roots were crushed by anelectrical grinderto make powder. The powder was then stored in an exceedinglyclean, dried and covered plastic container at room temperature, andstored until used.

Preparation of extracts

A hundred grams of root powder was added to 900 ml of either 90% ethanol or boiled distilled water to prepare two extracts. The obtained concentrates were reconstituted in ethanol orboiled distilled water (1:10 w/v), respectively. The mixtures were covered and shaken every 30 minutes for six hours, then left to stand for 48 hours for extraction. Then, the mixtures were filtered through filter paper and 200 mL of 90% ethanol or boiled distilled water was added. The two extraction methods were repeated twice. The obtained extracts were then evaporated at (50°C) using a rotary evaporator, and alsothe resulting crude extracts were dried using a desiccant. Dried extracts were collected, weighed and stored frozen at (-20 °C) until usedforthe analysis (Ezeet al., 2013).

Methods

All chemical analysis of extracts was done in the Food Safety and Quality Control Lab, in the Faculty of Agriculture, Cairo University, Giza, Egypt.

Chemical composition

Determination of moisture, proteins, fats, ash, crude fiber contents in extracts was according to AOAC. (2010). Protein content was determined by Kjeldahl, using a factor of 6.25, carbohydrates were calculated by difference:

Total carbohydrates = 100 - (g protein+ g fat +g ash + g fiber).

Total Carotenoids

Extracts were reconstituted in 1 to 10 mL of acetone and solicited for 2min. Visible spectra (340 to 700 nm, 1 nm interval) were collected using a 1-mL quartz cuvette in a UV/Vis. Spectrophotometer, Jenway, England according to Biehleret al., (2010).

DPPH Radical-Scavenging Activity

0.1 g of each sample was prepared in 50ml methanol. An aliquot of the extract was added to DPPH radical (100 µl, 0.2 mM) dissolved in methanol. The mixture was stirred and left to stand for 15 min in dark. Then the absorbance was measured at 517 nm against a blank. Percentage scavenging effect was calculated as: [(A0 – A1) / A0] × 100 where: A0 is the absorbance of the control (without sample) and A1 is the absorbance in the presence of the sample (Brand-Williamset al., 1995).

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Vitamin C

Oneg ofeach sample was extracted with 100 ml of the oxalic acid - EDTA solution. The extract is filtered through a filter paper then centrifuged. A 5-ml aliquot is transferred into a 25-ml calibrated flask and mixed with other reagents 0.5ml of the metaphosphoric acid-acetic acid solution and 1 ml of 5% V/V sulphuric acid, followed by 2 ml of ammonium molybdate reagent. After 15min measure the absorbance at 760 nm against a reagent blank by Brubacheret al., (1985).

Phenols HPLC

Agilent1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, aKinetex®5µm EVO C18 100 mm x 4.6 mm, (Phenomenex, USA), operated at 30oC. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.2 % H3PO4 (v/v), (B) methanol, and (C) acetonitrile. The injected volume was 20 µL. Detection: VWD detector set at 284 nm as described (Agilent Application, 2014).

Statistical analysis

Statistical analyses were carried out in triplicates using one-way ANOVA and alsothe data were expressed as mean ± Standard deviation at the degree of confidence limit p value < 0.05.

Results and discussion

Table (1) showed the approximate chemical composition of dried costus root powder of boiledaqueous and ethanolic extracts.

Table (1): approximate chemical composition of dried costus root powder of boiled aqueous and ethanolic extracts

Compounds	Boiled aqueous	Ethanol 90%
Ash	1.57±0.03	1.51±0.02
Fiber	0.04±0.00	1.14±0.01
Total Protein	2.74±0.07	4.37±0. 08
Total lipids	5.74±0.17	9.57±0.18
***Carbohydrate**	89.94±0.01	83.03±0. 61

***Carbohydrate was calculated by difference.**

The results in Table 1 showed that higher amounts of total protein and total lipids were obtained using ethanol for extraction, whose values in ethanol extract were twice those values in boiled aqueous extract (4.37& 2.74 and9.57& 5.74 g/100 g), respectively. A lower amount of fibre was also observed in the boiled aqueous extract compared to the ethanol extract. The carbohydrate content of the boiled aqueous extract (89.94%) was higher than that of the ethanol extract Moreover, in extracts, ethanol and water, close ash contents were observed.

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Table (2) showed DPPH radical scavenging activity (%) in a dose-dependent manner presenting a maximum effect at 1.0% of the concentration of dried costus root powder of boiledaqueous and ethanolic extracts.

Table (2): radical scavenging activity % of dried costus root powder of boiled aqueous and ethanolic extracts

Extract conc	Boiled aqueous	Ethanol 90%
1.0%	90.12±0.24	95.44±0.64
0.5%	89.36±0.05	89.75±0.08
0.2%	88.44±0.11	82.66±0.06

The data in Table 2 showed that both aqueous and ethanolic S. lappa showed high DPPH. The antioxidant activities of S. lappa increased in the test regimen in a concentration-dependent manner.Similarly, Chang,et al., 2012 declared thatS.lappademonstrateda strong DPPH radical scavenging activity (92.98%)and increased with increasing extract concentration. They noted thatS. lappa root, used as an alternative antioxidant agent in the medical and food industry, proved that the toxicity associated with high concentrations is resolved in future studies

The results were found to be consistent with those reported by Elgharabawyet al. (2021) who found that ethanolic costus root extract has highradical scavenging and antioxidant activities.

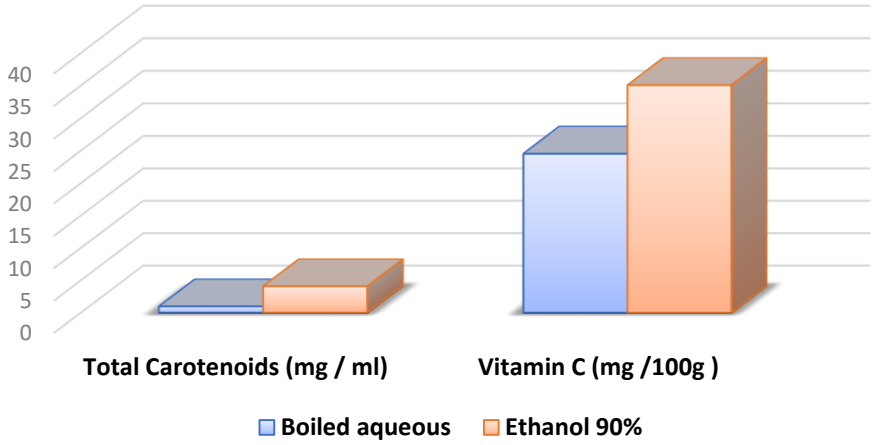
The increase of DPPH scavenging activity with increasing extract concentration is alsoin harmony with those findings of Lee& Kang (2020) and Alotaibi,et al., (2021). As free radicals are important mediators that provoke or sustain inflammatory responses, and their neutralization by antioxidants and radical scavengers can reduce neuroinflammation, DPPH radical assay is one of the generally used methods for calculating the free radical scavenging activities of antioxidants (Sachindra, et al., 2010).

Total carotenoids and vitamin C content of dried costus root powder of boiledaqueous and ethanolic extracts are shown in Figure (1).

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Fig 1: Total carotenoids and vitamin C content of dried costus root powder of boiled aqueous and ethanolic extracts



The results illustrated in figure 1 revealed that total carotenoids and Vitamin C contents of ethanol extract of costus root powder were muchhigher compared to boiled aqueous extract.

These results are found to be in harmony with those reported by Naseer,et al., (2022) demonstrating that plant methanolic and chloroform extractsshowed positive results for flavonoids, alkaloids, proteins, β-carotene, lycopene,coumarins, saponins, carbohydrates, fats, and quinones.

These phytochemicals have multi-functional properties; carbohydrates have water holding capacity, binding of toxins, and bile acid, and phenols, polyphenols, flavonoids, and carotenoids are antimicrobial, anti-inflammatory, anti-tumor, and antioxidant (Abbasi et al., 2020, Iqbal et al., 2020, Iqbal et al., 2021, Abbasi et al., 2021, Uddin et al., 2021).

The quantitative phytochemical analysis revealed that on average some extracts of plant parts had high phenolic, flavonoid, β-carotene, and lycopene content. The high phenolic compounds contribute to the enhanced ability of plants to quench reactive oxygen species (ROS) due to their redox potential (Singh and Chahal, 2018).The plant proved to contain small amounts of various essential vitamins such as vitamin A, vitamin B_1, vitamin B_2, vitamin B_{12}, and vitamin C. (Mishraet al., 2013).

Medicinal plants are highly rich in bioactive phytochemicals and nutraceutical compounds such as alkaloids, flavonoids, terpenoids, tannins and saponins, vitamins, proteins (Iqbalet al., 2019).

Flavonoids profile screening of dried costus root powder of boiled aqueous and ethanolic extracts are illustrated in table 3.

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Table (3): Flavonoids profile screening of dried costus root powder of boiled aqueous and ethanolic extracts (mg\kg)

Constituent	Boiled aqueous	Ethanol 90%
Rutin	249.41	324.13
Myricetin	116.09	427.41
Quercitin	141.71	-
Neringein	77.58	-

The phytochemical screening of extracts revealed the presence of flavonoids compounds in root extracts of Saussureacostus (Table 3). In the boiled aqueous extract, the four flavonoid compounds were Rutin, Quercitin, Myricetin, and Neringein in quantitatively descending order.
In the ethanolic extract, Myricetin was the major component (427.41 mg\kg). Following it, Rutin showed 324.13 mg\kg, while Quercitin, and Neringein were absent.

In this respect, Hend (2022) found two flavonoids (Quercetin and Rutin) in costus roots. Flavonoids are a class of natural phenolic compounds synthesized in plants as bioactive secondary metabolites (Nabaviet al., 2020) that are responsible for the characteristics of flavor, color, and pharmacological activities (Scarano et al., 2018). They are potent antioxidants protecting plants from unfavorable environmental conditions (Nabaviet al., 2020). Studies also have shown that flavonoids have immunomodulatory, anti-inflammatory (Yahfoufiet al., 2018), and anticancer activities (Rodriguez-Garcia and Sanchez-Quesada, 2019).

Quantitative HPLC and chromatograms of dried costus roots powder are presented in Figure 2 for boiled aqueous extract.

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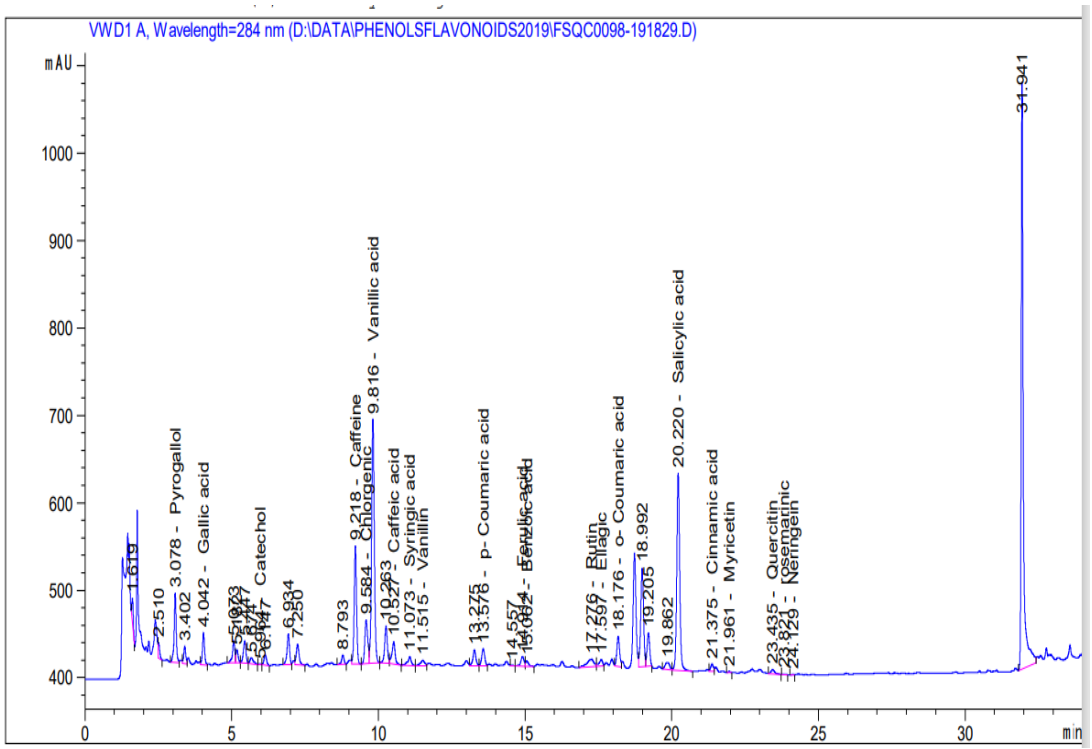


Fig. 2: HPLC Chromatograms of phytochemical screening of dried costus root powder of boiled aqueous extracts

The results presented in figure 2 showed that boiled aqueous extract of Costus roots was found to be extensive in Salicylic acid, Vanillic acid, Ellagic, Caffeine, Rutin, Benzoic acid, Pyrogallol, Quercitin, Chlorogenic, and Myricetin, respectivelydescendingly.

These results demonstrated uniformity with Hashimi,et al., (2020), the introductory phytochemical screening of the aqueous extracts showed the presence of several phytoconstituents. These bioactive agents can act as anti-inflammatory, hepatoprotective, antioxidant, and anticancerous agents. These properties may be the reason for their ethnomedical use in many diseases identified in classical literature.

As Phenolic and Flavonoid compounds are the most antioxidants that prevent oxidative cell damage,beinganti-inflammatory, anti-thrombotic, and anti-allergic (Uchegbu,et al., 2016). The contents of phenolic compounds in Costus roots powder extracts were determined, and presented in Table (4).

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Table (4): Phenols profile screening of dried costus root powder of boiled aqueous and ethanolic extracts (mg\kg)

Constituent	Boiled aqueous	Ethanol 90%
Pyrogallol	216.08	1027.80
Gallic acid	57.08	442.38
Catechol	4.50	-
p-Hydroxy benzoic acid	-	124.88
Caffeine	465.50	2295.47
Chlorgenic	127.95	118.88
Vanillic acid	1188.72	3015.06
Caffeic acid	38.67	156.08
Syringic acid	23.50	82.75
Vanillin	16.09	170.38
p-Coumaric acid	25.82	64.78
Ferulic acid	22.99	102.31
Benzoic acid	223.13	956.42
Ellagic	641.55	-
o-Coumaric acid	35.04	103.43
Salicylic acid	2360.63	8307.96
Cinnamic acid	6.69	4.85
Rosemarinic	27.80	-

The predominant phenolic compounds were Salicylic acid and Vanillic acid in both extracts. Cinnamic acid was found in the low concentrations for both extracts.

The total content of phenolic compounds in Costus root powder extracts was correlated with the solvent used, where there was a high amount of phenol in ethanol extract for example (8307.96 mg\kg as Salicylic acid), compared to in boiled aqueous extract (2360.63 mg\kg as Salicylic acid).

The ethanol extract also contained higher values of other phenolic compounds when compared with boiled aqueous extract, as reached 3015.06, 2295.47, 1027.80 442.38, and

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64.78 mg\kg for Vanillic acid, Caffeine, Pyrogallol, Gallic acid, and p-Coumaric acid, respectively. However, Catechol, Ellagic, and Rosemarinic were found only in the boiled aqueous extract recording (4.50, 641.55, and 27.80 mg\kg, respectively).

In aqueous extract for Costus roots,Eleven phenolic acids (gallic, p-Hydroxy benzoic, vanillin, Caffeic, syringic, p-Coumaric, Ferulic, Benzoic, o-Coumaric, Salicylic, and Cinnamic), and 7 Phenoliccompounds (Pyrogallol, Catechol, Caffeine, Chlorgenic, Vanillin, Ellagic, and Rosemarinic) were identified as standard.

Figure (3) depicted the HPLC Chromatograms of S. lappa root extracts of ethanol extract.

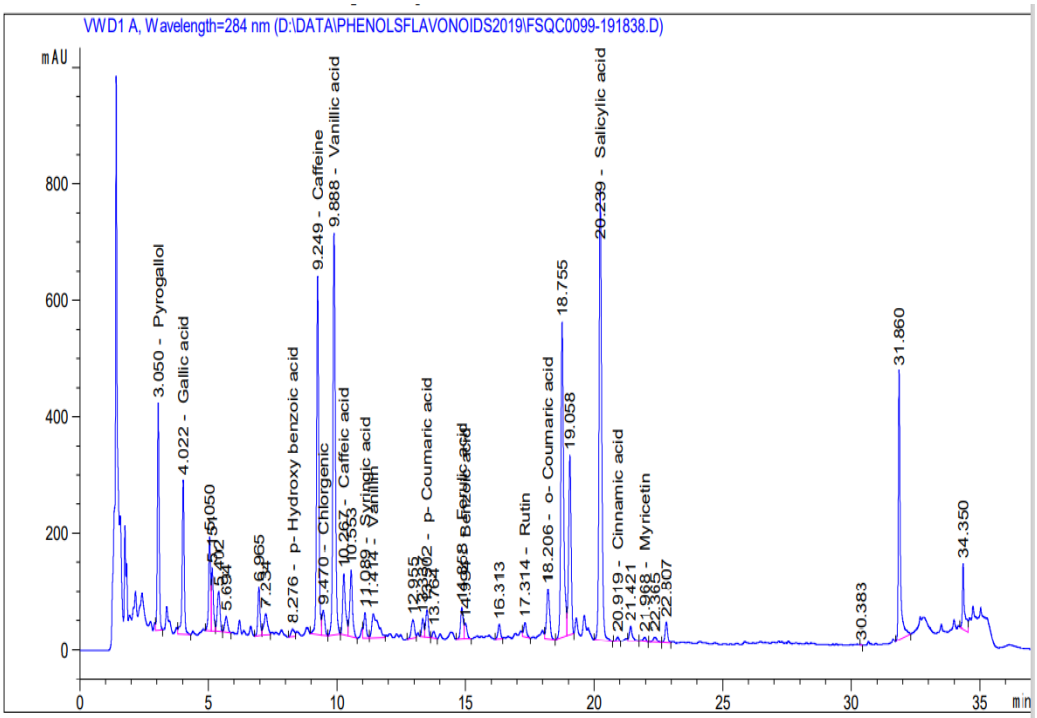


Fig. 3: HPLC Chromatograms of phytochemical screening of dried costus root powder of ethanolic extracts

The datain figure (3) indicated the presence of phenolic compounds in both two extracts of S. costus with different concentration levels. Ethanol extract had the higher contents of polyphenols. The highest detected phytochemical compounds are found in order; Salicylic acid, Vanillic acid, Caffeine, Pyrogallol, Benzoic acid, Gallic acid, Myricetin, and Rutin, followed by other compounds. Moreover, p-Hydroxy benzoic acid was found in ethanol extract. However, Quercitin, Neringein, Catechol, Ellagic, and Rosemarinic were not detected in the extract.

Polyphenol compounds are bioactive compounds commonly found in plants that are consumed by humans. Polyphenols possess antibacterial and antifungal activities (Gutiérrez-Venegas, et al., 2019). In addition, Karthikeyan et al., (2012) reported that the ethanol extract of costus possesses a higher amount of phenolic compounds than the aqueous extract.

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The contents of phenolics and flavonoids were examined under the Folin-Ciocalteu's colorimetric showed the strongest inhibitory potential on 2,2-diphenyl-1-picrylhydrazyl (DPPH), and thus demonstrated their anti-oxidative effectiveness (Chang et al., 2012). Further, El-Salam et al., (2019) reported that phytochemicalsfrom plants are being used for the prevention of various diseases mainly caused by free radicals; the higher polyphenol content would then exhibit stronger inhibition and also higher antioxidant activity.

In this respect, Waly, (2009) suggested that costus as a blood purifier, antiseptic and to increase the cetaceous circulation, a good insect repellant, internally it is a good expectorant, anti-spasmodic, and neurotoxin, and therefore, can be used for cough, bronchitis, bronchial asthma, paralysis, facial palsy, and neurasthenia.

The findings of Gwari, et al., (2013), and Negi, et al., (2013) indicated that S. lappa and S. costus are rich in the phyto constituents where gas chromatography-mass spectrometry analysis and their ethanol extracts resulted in the identification of 39 and 41 compounds, respectively, with sesquiterpenes as a major component. Further, Omer, et al., (2019) confirmed that S. lappa root is rich in various bioactive compounds as indicated in the gas chromatography-mass spectrometry profiles of the ethanol and aqueous extracts. The gas chromatography-mass spectrometry analysis has led to the identification of a total of 37 and 18 compounds in the ethanol and aqueous extracts, respectively illustrating that ethanol extract of S. lappa has almost double phyto constituents as compounds identified in the aqueous extract.

Recently Bolkiny, et al., (2019) confirmed that either hypothyroidism or hyperthyroidism in male mice was associated with biochemical indices alterations and that treatment with costus also attenuated these changes in blood indicating the attenuating therapeutic effect of costus during Thyroid disorders. Therefore, they proposed that the extract of costusroots can be used as an adjuvant co-therapy in hypothyroid and hyperthyroid syndromes.

Similarly, Semwal, et al., (2020) observed that S. costus has the potential to treat many human diseases including respiratory, gastrointestinal disorders, and various metabolic disorders. Thus, they concluded phyto chemicals may be responsible for the biological activities of S. costus.

Conclusion

The phytochemical screening of ethanolic and aqueous extracts ofS. lappa root proved the presence of various bioactive compounds in both extracts, i.e. flavonoids, Phenols, proteins, carotene, carbohydrates, fats, and vitamin C. The ethanol extract showed more quantitative of some bioactive compounds than those for aqueous extract. Since the antioxidant activities are related tothe bioactive compounds, it is expected that the root of S. lappa, as well as its extracts, can be used as an alternative prophyl acticantioxidant agent. The S. lappa extractscan play a great role in the defensive action against the human multi-resistant pathogens and alternate the antibiotics in treating some infections. These extracts could be used in the food industry as an alternative antioxidant for traditional chemical preservatives

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that threaten human health.It is worth separating and identifying these bioactive compounds from the roots of S. lappato obtains new natural and effective compounds. Other future studies have been proposed using different solvents and extraction systems and assume that there are perhaps more bioactive compounds. More future research needs to be done on interesting phyto chemicals as well as isolation, and purification techniques in addition to their applications in some food products.

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