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# Ceramide from *Celocia argentea* Leaves by LC-MS

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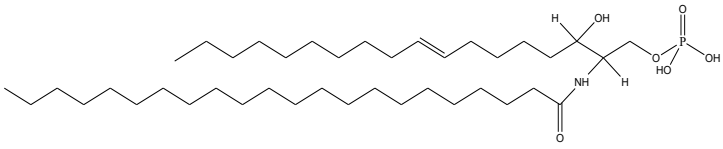
**ABSTRACT**

Celocia argentea, a traditional medicinal plant belongs to family Amaranthaceae is well known for its antioxidant, hepatoprotective, cytotoxic, immunomodulating, antidiabetic activities. The dried powder of C. argentea leaves was extracted in $CH_3OH$:$H_2O$ (1:4) v/v, concentrated to its $1/10^{th}$ volume. The exhaustive analysis of C. argentea leaves extract was separated and characterized using HPLC-MS-Q-TOF. The structures were studied and proposed by ESI-MS/MS with MassHunter software. The CerP(d18:1/22:0), is ceramide with phosphate identified at m/z 722.5232 and at retention time 12.03 min is due to adduction of potassium ion followed by loss of water molecule having combination of elements is $C_{40}H_{80}NO_6P$ shows 1.94 ppm error. Present study will support in future to propose the plant as a good source of lipid, which will support as a dietary supplement and can be used as a component of cosmetic materials. Apart from this there are compounds of biological origin are the medicines used for chronic as well as severe diseases like cancer, tuberculosis etc.




**Keywords:** Celocia argentea, HPLC-MS-Q-TOF, Ceramide, Herbal medicine

## INTRODUCTION

*Celocia argentea* L. commonly known as Cockscomb, Lagos spinach, is weed found worldwide belonging to Amaranthaceae family. It is traditional medicinal plant in Asian country commonly used by rural population. Indian origin *C. argentea* have more than sixty varieties over the world. The entire plant is utilized, and some of its benefits were reported by tribal people in folk medicine. Historically, this plant has been widely used, the composition of its seeds being more studied than that of leaves. Leaves are applied in traditional medicine for treatment of eczema, diarrhea, and throat. The secondary metabolites, glycosides isolated from seed are used as skin whitening agent and for strengthening of eyes. The plant is used to cook in the time scarcity as a leafy vegetable. Recently, the secondary metabolites isolated from the seeds have been reported for various biological activities. Seed from *C. argentea* are rich in betalains which confer antioxidant activity. Isolated flavonoids from seed are responsible for good antioxidant, hepatoprotective, cytotoxic, immunomodulating, antidiabetic activities [1,2]. *C. argentea* leaves has relevance for developing dietary role due to valuable phytochemical carbohydrates, vitamins and essential macro and micronutrients.

There is need to investigate bioactive phytoconstituents for developing nutraceuticals and dietary supplements. A prerequisite for investigating bioavailability and biochemical effect of any dietary phytochemicals is to know the occurrence of metabolites group in a given plant species with their qualitative and quantitative composition. In agricultural and food science the metabolism is very important growing field of analytical chemistry for identification of small metabolites present in small plant tissues [3]. Metabolite profiling is the analytical method for relative quantification of number of metabolites from biological samples, restricting itself to a certain range of compounds or event to screening a pre-defined number of members of compounds of class [4]. Thus, mass spectrometric methods are most widely used in the field of metabolomics, LC-DAD-MS with electron spray ionization (ESI) being used increasingly for screening botanical metabolites [5,6].Identification of phenolic acids, lipids and other polar phytoconstituents from *C. argentea* has been usefully performed by RP-HPLC coupled with mass spectrometry. Highly efficient resolution and characterization of wide range of polar compounds is achieved for separation of compounds by polarity differences in LC-MS hyphenated technique [7]. The mass accuracy and true isotopic pattern provided by Quaternary Time of Flight-Mass Spectrometry (Q-TOF-MS) for both precursor and fragment ions enabled the determination of many well-known compounds present in *C. argentea* by facilitating additional information key for determining the elemental composition.

The aim of this study is to present a complete exhaustive analysis of *C. argentea* leaves extract. HPLC-MS-Q-TOF was used for characterization and identification of untargeted phytochemicals covers wide mass range and target many compound classes, representing overall richness of plant. Extraction program was performed as per the guidelines for analysis of phytochemicals by J. Horborne. The structures were studied and proposed by ESI-MS/MS with MassHunter software [8]. In future these bioactive can be isolated and analyze quantitatively will be useful for various biological activities and formulations of new herbal medicine. LC-MS detects the large group of plant secondary metabolites such as alkaloids, saponins, phenolic acids, phenyl propanoid, flavonoids, glucosinolates, polyamines; depending upon type of stationary phase used [9].

Actually, from early human history, natural products from plants have always been investigated and utilized to treat numerous diseases. Medicinal plants are productive sources to provide large number of bioactive molecule, which could be screened to find potential lead compound for drug discovery [10]. The efficacy, safety and quality of phytomedicine are the important need for therapeutic purposes. Among all the identified hundreds of phytoconstituents, only a few constituent are bioactive, means having potential for biological activity. Hence it is necessary to identify and measure all the bioactive phytoconstituents of medicinal plant to ensure the reliability and repeatability of clinical research and enhance the quality from the pharmacological beneficial and or hazardous perspectives. Because of complexity and

variability of botanical extracts, it has presented significant challenges for separation and detection methods enabling rapid analysis of the chemical composition of medicinal plant. Identification and quantification of trace metabolites of natural products from complex biological matrices requires sophisticated analytical methods with high sensitivity and selectivity.

## METHODOLOGY

The analysis of extracted sample was carried out on LC-MS-Q-TOF spectrometer for metabolite profiling of *C. argentea* leaves powder using the solvent gradient elution method. Liquid chromatography is coupled with high resolution tandem mass spectrometer, has greatly facilitated explicit identification and highly sensitive quantification of trace component present in complex matrices, which can accomplish qualitative analysis in short time. The present study summarizes recent developments of identification and measuring phytochemical constituents and their metabolites, as well fingerprinting analysis for *C. argentea* are highlighted. Metabolite profiling of phytochemical constituents from matrices helps to confirm known compounds and structure of unknown compounds. After profiling phytoconstituents need to separate chemically and followed by bioassay for new active chemical entity from natural sources. Significance of the study of metabolite profiling is still growing due to their numerous functions in plants as well as their beneficial effects on human health. Identification of phenolic metabolites presents in complex plant extracts is an important task during studies in many areas of biomedical research. Secondary metabolites present in complex plant extracts are often isomeric or isobaric compounds, with different substituents to parent compound shows very similar chromatographic properties. These phytochemical properties results in co-elution of numerous metabolites due to lack of resolving power of HPLC column. The substituents such as sugar moieties, consisting of simple hexoses and pentoses (glucose or xylose), deoxysugars (rhamnose) or sugar acids (glucuronic acid), acyls (aliphatic and aromatic acids) and alkyls, demands for a usage is special analytical techniques for identification of secondary metabolites. There is need to more developments on isolation and purification of phytochemical from natural product extracts, which is laborious, tedious and time consuming to identify the compound by earlier IR, MS and NMR techniques are known (dereplication) or uninteresting. Now LC-MS can rapidly identify known or unknown compounds using fragmentation pattern of molecular species.

In leaves of *C. argentea*, all the compounds are verified in terms of retention time, accurate mass measurement data, elemental composition and error in ppm by comparing the results with those in literatures and a number of established database of LC-ESI-Q-TOF-MS from exact mass measurement. The data were processed using MassHunter® software in Agilent Q-TOF-MS.

**Extraction *C. argentea* leaves for LC-MS**

Prior to LC-MS analysis of secondary metabolites, sample preparation is regarded as an obligatory procedure because extracting the desired chemical component from the herbal materials is the important first step in analytical process. The popular soxhlet extraction technique is used for the extraction, clean-up, and concentration of analytes from different matrices can effectively reduces matrix effect which has significant impact on the accuracy, precision and robustness of bio-analytical methods.

The extract procedure was carried out as per the standard methods of phytochemical analysis. The dried powder of *C. argentea* leaves was extracted in $CH_3OH$:$H_2O$ (1:4) v/v, the extract was concentrated to its $1/10^{th}$ volume. The resultant extract was acidify using 2M HCl and extracted with chloroform. The chloroform layer was concentrated in vacuum. The aqueous layer was excluded from further study. The LC-MS-Q-TOF analysis performed and tentatively identified compounds is highlighting retention time, elemental composition, precursor ion, m/z, exact mass, error and identification of metabolite. Ceramides- drug of biological origin were unambiguously identified, and tentative structures were proposed for compounds.

## RESULTS AND DISCUSSION

Ceramides contain fatty acids linked by an amide bond to the amine group of long chain base. They play an important role for biosynthesis of the complex spingolipids and play role for cellular signaling, regulation of apoptosis, cell differentiation, transformation and proliferation. They are esterified to terminal hydroxyl group, prevents the loss of moisture through the skin. The glycoceramides found in skin of plant and animal tissues contain basic ceramide unit linked by a glycosidic bond by a carbon 1 of the long-chain base to glucose or galactose, which were reported in brain lipids called as cerebroside. They are act as biosynthetic precursor of lactoceramide and are part of water permeability barrier.

The CerP(d18:1/22:0), **cpd**-406 is ceramide with phosphate identified at m/z 722.5232 and at retention time 12.03 min is due to adduction of potassium ion followed by loss of water molecule having combination of elements is $C_{40}H_{80}NO_6P$ shows 1.94 ppm error. The dihydroceramide C2, **cpd**-169 appeared on chromatogram at m/z and retention time 326.3053 and 8.93 min respectively due to protonation followed by loss of water molecule possesses $C_{20}H_{41}NO_3$ elemental combination with 0.28 ppm error. The glucosylceramide, cpd-**366** and cpd-**536** are the ceramide unit is linked to glucose appeared on chromatogram at 11.49 and 15.40 min for m/z to molecular ion is 72.536 and 661.536 are caused due to adduct of sodium and ammonium ion have elemental combination is $C_{40}H_{77}NO_8$ and $C_{36}H_{69}NO_8$ with 2.6 and 0.4 ppm error. The cerebroside C **cpd**-515 is identified at m/z of molecular ion is 771.6082 and at retention time 14.64 min caused due to adduction of ammonium ion form molecule, having elemental combination is $C_{43}H_{79}NO_9$ with only 0.4 ppm error.

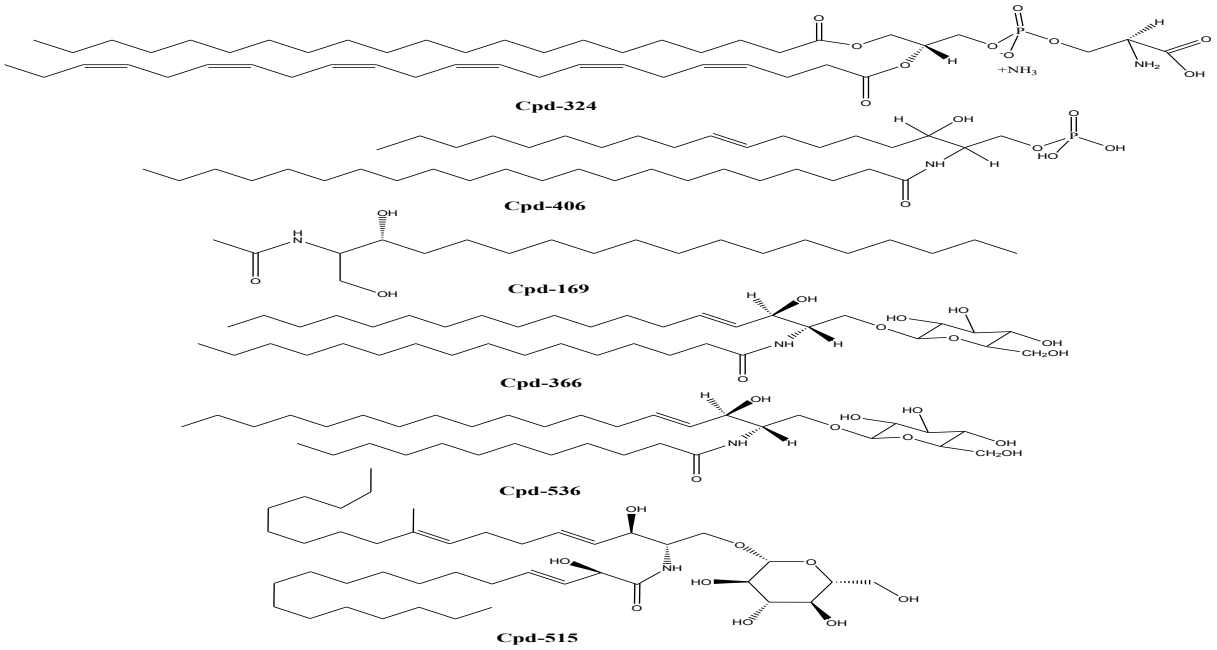



**Figure:** Structures of phytochemical metabolites- Ceramide

## CONCLUSION

The qualitative analysis of *C. argentea* leaves extract performed with HPLC-MS-Q-TOF identify the presence Ceramides were identified, and tentative structures were proposed for compounds. Globally, phytomedicine manufacture needs to maintain the purity of extract from active phytoconstituents quote active marker compound for quality of active extracts.

Present study will support in future to propose the plant as a good source of Ceramides as a lipid, which will support as a dietary supplement and can be used as a component of cosmetic materials. Apart from this there are compounds of biological origin are the

medicines used for chronic as well as severe diseases like cancer, tuberculosis etc.

In this work the extensive qualitative identification of phytochemical metabolites using HPLC-Q-TOF-MS method was employed for quality determination of herbal medicinal products and formulations containing *C. argentea* leaves have been established. The extract may play significant role in prevention of degenerative diseases**.** Ceramides of biological origin have been identified using literature survey, providing the first comprehensive characterization available on the phytochemical composition of the leaves of *C. argentea,* highlighting it is an abundant source of antioxidant phenolics and phytochemicals. The result obtained may develop the current knowledge on *C. argentea*, boost further research toward exploring of bioactive compounds and may encourage more consumption of this important functional food.

**Conflicts of interest:** The authors stated that no conflicts of interest.

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