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# Phytochemical Analysis and Evaluation of the Medicinal Herb, *cosmos caudatus kunth.* for Invitro Antioxidant Activities

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**Abstract**: *Cosmos caudatus kunth belongs to the family asteraceae and is also a popular garden plant. It is an annual herb 1-8 ft tall. Leaf stalks are 1-7 cm long, leaves are 10-20 cm long and dissected. This plant is supposed to be edible. It mostly flowers from June to November. Cosmos caudatus has been spread to many countries over the world indicating that it has a beneficial attribute. Despite this C.caudatus is only being used in traditional and alternative medicine. The aim of this review is to analyse qualitative and quantitative phytochemical analysis of this species and evaluate in vitro antioxidant properties of cosmos caudatus kunth. From this study it can be concluded that the species of cosmos caudatus it exhibits the presence of alkaloids, total phenolics total flavonoids, tannins, saponins in considerable quantity and the species also has an effective potential to be a powerful antioxidant.*

**Keywords**: *Cosmos caudatus,* Phytochemical Analysis, Invitro Antioxidant Activities, etc.

## I. INTRODUCTION

*Cosmos caudatus* is also called as ulam which refers to a group of traditional Malaysian vegetables, and it is usually consumed in raw form. This plant has always been one of the great sources of medicinal and traditional practices, for a centuries all over the world for treatment of various human diseases. In Malaysia the ministry of health has been promoting the consumption of cosmos under the Malaysian Dietary guidelines (2010). After the survey it was revealed that salad and raw vegetables consumers tend to have increase serum level of vitamin c, vitamin E, folic acid, B-carotene and lycopene. Traditionally, *C.caudatus* has been used to boost blood circulation, to strengthen the bones, to reduce body heat also as an anti-agent to treat infectious disease.

In addition, various studies have revealed that *C.caudatus* has potential medicinal properties with the increasing interest in the traditional medicinal plants and herbs. There is need to review the traditional claims about *C.caudatus* with regards to its medicinal uses before it can be used for further neutraceutical applications. However no much scientific validation has been made for this species for its medicinal uses. To address this lacuna, the present study was carried out for qualitative and quantitative phytochemical analysis and in vitro antioxidant activities of leaf and root parts in *cosmos caudatus* using various alcoholic (Petroleum ether, chloroform, ethyl acetate and methanol) and aquous extracts.

## II. MATERIAL AND METHODS

Plant samples were collected from the fields and fresh leaves and root parts of this species were washed under running tap water, dried in shade at room temperature and were made in powdered form.

### Extract Preparation

The powdered plant samples (50g/250 mL) were extracted successively with petroleum ether, chloroform, ethyl acetate, methanol and water using soxhlet apparatus at 55-85$^0$C for 8-10 h in order to extract the polar and non-polar compounds Elgorashi et al (2004). For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were reduced under room temperature and stored at 4$^0$ C for further use.

The dried plant extracts were then redissolved in dimethyl sulfoxide and to get the solution of 10 mg/ 10mL. For each extract which was subjected to analysis of in vitro antioxidant activities.

Quantitative phytochemical analysis

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**Determination of Alkaloids**

A total of 200 ml of 20% acetic acid was added to 5g of leaf and root powders taken in separate 250 ml. beaker and covered to stand for 4 hrs. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample concentrated ammonium hydroxide was added drop wise until the precipitate was complete and whole solution was allowed to settle and precipitate was collected by filteration and weighed B. O. Obadoni et al (2001). The percentage of total alkaloid content was calculated as:

Percentage of total alkaloids (%) = Weight of residue × 100 / Weight of sample taken.

**Total Phenolics Content**

The total phenolics content of *Cosmos caudatus* was estimated using Folin – Ciocalteau reagent by the method of Sidduraja and Becker (2003). About 20 µg of leaf and root extracts were taken separately and it was made up to 1mL with distilled water. Then 500 µL of diluted Folins- phenol reagent (1:1 ratio with water and 2.5 mL of sodium carbonate (20%) were added. The mixture was shaken well and incubated in dark condition for 40 min for the development of colour. After incubation the absorbance was measured at 725 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 µg/mL. The total phenolics content in the plant extracts were expressed as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve.

**Total Flavonoids Content**

The total falvonoid content was estimated using the procedure described by Zhishen et al (1999). A total of 1 ml of plant extracts were diluted with 200µL of distilled water separately followed by the addition of 150 µL of sodium nitrite (5%) solution. This mixture was incubated for 5 min and then 150µL of aluminium chloride (10%) solution was added and allowed to stand for 6 min. Then 2 mL of sodium hydroxide (4%) solution was added and made up to 5 mL with distilled water. The mixture was shaken well and left it for 15 min at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavanoids content. The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

**Estimation of tannin content**

Tannins content of *Cosmos caudatus* was estimated by the method of Siddhuraj and Manian (2007), a total of 500 µL of the extracts were taken in test tube separately and treated with 100 mg of polyvinyl polypyroolidone and 500 µL of distilled water. This solution was incubated at $4^0$C for 4 hrs. Then the sample was centrifuged at 5000r/min for 5 min and 20µL of the supernatant was taken. This supernatant has only simple phenolics free tannins. The phenolics content of the supernatant was measured at 725 nm and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannins content of the extract was calculated as follows:

Tannins (mg GAE/g extract = Total phenolics (mg GAE/g extract)- Free phenolics (mg GAE/g extract)

**Estimation of Total Saponins Content**

Estimation of total saponins content was determined by the method described by Makkar et al (2007). based on vanillin - sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol) was added. Then 2.5 ml of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at $60^0$C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard curve.

**Invitro Antioxidant Activities**

*C.caudatus* has been reported to have antioxidant activity potential. An experiment was conducted where raw vegetables extracts were gained using different solvent systems 70% acetone, 70% methanol, 70% ethanol and distilled water. Highest flavonoid content was gained from 70% methanol extract of *C.caudatus* (27.7 ± 1,0 mg QE/g dry weight basis )

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S.F.Sulaiman et al (2011) . This is expected to have positive effects for degenerative diseases prevention D.P. Makris and J.T. Rossiter, (2002).

## III. RESULTS

### Qualitative Phytochemical Analysis

The present study revealed that the various alcoholic and aqueous extracts of leaf and root parts of *C.caudatus* contained alkaloids, cardiac glycosides, terpenoids and triterpenoids( Table 1 ). However, phenols were detected only in methanolic extracts of both parts and the cardiac glycosides were found in root extracts of the solvents chloroform, ethyl acetate and methanol. Next to methanol extract, ethyl acetate extracts of both parts showed the presence of rich variety of secondary metabolites. Petroleum ether, chloroform and water extracts showed the less variety of these secondary metabolites. Compared to all other solvent extract, methanolic leaf and root extracts had higher number of secondary metabolites with high degree of precipitation (√ √ √). Triterpenoids and resins were determined to be present with lesser amount (√) only in all extracts.

**Table 1:** Preliminary qualitative analysis of various alcoholic and aqueous extracts of leaf and root parts of *C.caudatus*

| Plant Constituents | Petroleum ether | | Chloroform | | Ethyl acetate | | Methanol | | Water | |
|---|---|---|---|---|---|---|---|---|---|---|
| | L | R | L | R | L | R | L | R | L | R |
| **Alkaloids** | × | × | √ | √√√ | × | × | √√√ | √√ | √ | × |
| **Cardiac glycosides** | √ | × | × | √√√ | × | √√√ | × | √√√ | √ | × |
| **Flavonoids** | × | × | × | × | × | √√√ | √√√ | √√ | √√√ | √√√ |
| **Glycosides** | × | × | × | × | √ | √ | √√√ | √√√ | × | × |
| **Phenols** | × | × | × | × | × | × | √√√ | √√√ | × | × |
| **Resins** | × | × | √ | √ | √ | × | √ | √ | √ | × |
| **Saponins** | × | × | √ | × | × | √√√ | √√√ | √ | × | × |
| **Tannins** | √ | × | × | × | √ | √√ | √√√ | √√ | × | × |

√√√ highly present, √√ moderately present, √ Low, × absent, L- leaf extract, R- root extract.

### Quantitative Determination of the Chemical Constituency

### Extraction Yield

Table 2 shows the percentage of yield of crude successive extracts (petroleum ether, chloroform, ethyl acetate, methanol and water) of leaf and root parts of *Cosmos caudatus* Methanolic extracts of root exhibited higher yield (30.06%) followed by methanolic leaf extract (19.08%). The ethyl acetate leaf extract showed the lowest yield of (0.50%). Water extracts of root and leaf also showed lower yields (2.73% and 1.46% respectively) followed by petroleum ether leaf extracts (5%). Petroleum ether and chloroform root extracts determined to have the same quantity of yield of 3.2% each.

### Determination of Alkaloids

The gravimetric analysis for total alkaloid contents in leaf and root parts of *C.caduatus* exhibited that higher alkaloid contents were present in leaf powder (4560.21 mg/100 gsample) than that of the root powder (3800.83 mg/100 g samples).

**Table 2:** Percentage yield, total phenolics, total flavonoids, tannins contents of various alcoholic and aqueous extracts of leaf and root parts of *C.caudatus*.

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| Sample | Percentage yield (w/w) | | Total phenolics | | Total flavonoids | | Tannins | | Saponins | |
|---|---|---|---|---|---|---|---|---|---|---|
| | L | R | L | R | L | R | L | R | L | R |
| PE | 5.00 | 3.2 | 0.77± 0.01 | 0.46± 0.01 | - | - | 0.17± 0.01 | 0.06± 0.02 | 15.73± 0.30 | 13.02± 0.06 |
| CH | 2.20 | 3.2 | 0.45± 0.07 | 0.71± 0.02 | - | 15.30± 0.03 | 0.12± 0.01 | 0.29± 0.03 | 14.01± 0.02 | 12.86± 0.02 |
| EA | 0.50 | 1.60 | 0.11± 0.04 | 0.44± 0.05 | 18.32± 0.09 | 16.34± 0.06 | 0.04± 0.01 | 0.18± 0.01 | 17.23± 0.01 | 17.28± 0.02 |
| ME | 19.08 | 30.06 | 3.75± 0.05 | 5.62± 0.03 | 8.84± 0.08 | 6.36± 0.07 | 1.03± 0.17 | 1.64± 0.09 | 13.46± 0.02 | 16.25± 0.02 |
| WA | 1.46 | 2.73 | 0.32± 0.01 | 0.70± 0.01 | 6.68± 0.11 | 3.61± 0.05 | 0.07± 0.01 | 0.18± 0.03 | 12.63± 0.02 | 13.40± 0.03 |

**Total Phenolics Content**

Total phenolics content of various extracts of leaf and root parts of *C.caudatus* was varying widely between 0.32 to 5.62 mg /100 g extract (Table 2. Methanolic extract of leaf and root parts were demonstrating higher total phenolics content (3.75 and 5.62 mg/ 100g extracts respectively than that of the other solvent extracts.

**Total Flavonoids Content**

The total flavonoid content was high in ethyl acetate leaf extract (18.32mg/ 100 g extract) followed by root chloroform and ethyl acetate extracts (15.30 and 16.34 mg/100 g extract respectively) (Table 2). In addition, it has noted that the flavonoids content was not deducted in petroleum ether leaf and root extracts and chloroform leaf extracts.

**Estimation of Tannin Content**

The tannin content of the various extracts of leaf and root parts of *C.caudatus* was determined to be ranged between 0.04 and 1.64 mg /100 g extract (Table 2). Among the solvents used the methanolic leaf and root extracts were registered high number of tannins 1.03 and 1.64 mg / 100 g extract respectively.

**Estimation of Saponins Content**

The total saponins contents of both leaf and root parts of *C.caudatus* were ranging between 12.63 and 17.28 mg/100 g extract across the various solvent extract studied (table 2). The ethyl acetate extract of leaf and root parts depicted high content of saponins 17.23 and 17.28 mg/100 g extract respectively.

**In vitro Antioxidant Activity**

The study evaluated methanol extracts of tropical plants for free radical scavenging activity with use of 1,1-diphenyl 1-2-picrylhydrazyl assay (DPPH). *C.caudatus* with 21.3 µg/ml showed the highest potential. It was also observed that *C.caudatus* extract behaved similarly to α-tocopherol or BHA. All of these findings support the traditional use of *C.caudatus* for antioxidant effects.

## IV. DISCUSSION

Preliminary qualitative phytochemical analysis made for the leaf and root parts of *C.caudatus* revealed the presence of alkaloids, cardiac glycosides, flavonoides, glycosides , phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids. These secondary metabolites are reported to have many biological and therapeutic properties Vishnu R et al (2013), so this species is expected to have many medicinal uses.

The extraction yield calculated for petroleum ether, chloroform, ethyl acetate, methanol and water extracts of both parts of *C. caudatus* showed that methanol extract registered higher percentage of yield. It may be due to high polarity of methanolic solvent which can draw high variety of plant constituents than the other solvents did Paulsamy and Jeeshna (2011). Generally, majority of the secondary metabolites studied and ascorbic acid in leaf and root parts of *C. caudatus* have present with higher amount in methanolic extract than that of the other alcoholic and aqueous solvents. The

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biological property, antioxidant activity was determined to be effective through various assays for the leaf and root parts of *C. caudatus.*

It was also found that *C.caudatus* possess the highest total amount of phenols among some vegetables with 1.52mg GAE/100g of fresh weight (Andarwulan, et al 2010). It is believed that phenol compounds are the main contributor of antioxidant activity in plant extracts. Rafat et al (2010) reported that the highest superoxide dismuyase assay activity and the highest free radical scavenging potential among five of the most popular Malaysian salad vegetables was obtained from *C.caudatus* its 86.85 % and 98.56 % extracts respectively DPPH is usually used as a substance to evaluate the antioxidant activity R Shah, H Kathad, R. Sheth, N. Sheth (2010).

In the present study, the extracts had significant scavenging effects on the DPPH radical which was increasing with the increase in the concentration of the sample from 50-250 µg/ml. Similar trend of DPPH free radical scavenging activity was already documented well R Vishnu (2013) and J Thambiraj (2012). On the basis of response in terms of scavenging radicals it is concluded that the species *C.caudatus* possessed potential antioxidant activity. It may be due to the presence of respective secondary metabolites such as phenolics, flavonoids and tannins and radical scavenging activity indicates that these phytochemical constituents are major contributers to the antioxidant potential of this species. Therefore, this species can be attempted to derive the drugs of antioxidant properties.

## V. CONCLUSION

From the above analysis it was confirmed that *C.caudatus* is an herb which has antibacterial, antifungal, antioxidant, property and therefore it is beneficial to human health. Lower doses of *C.caudatus* are considered as safe to be consumed. Further studies on active compounds of this herb are strongly recommended in order to determine the substance responsible for its effects. This is a basic premise for future research and potential industrial use of this herb to treat diseases such as blood pressure issues, arthritis anticancer and bone strength agents. If this view get success the market can have a new powerful drug with variety of uses in distant future.

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