Antibacterial, antifungal and antioxidant activity of *Cleome coluteoides*: An In Vitro Comparative Study between Leaves, Stems, and Flowers

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Abstract

Objective: *Cleome coluteoides* belongs to Capparidaceae family and has been used in folk medicine for a long time. The aim of our research was measuring the antioxidant, antibacterial and antifungal activities of *C. coluteoides.*

Materials and Methods: different parts of plant were extracted by various solvents (ethyl acetate, methanol and dichloromethane). Antibacterial and antifungal activities were assayed by disk and well diffusion methods. The antioxidant activity was screened by DPPH and FRAP methods.

Results: The results showed that Gram negative bacteria and fungus were resistant to various plant extracts. Flower extract of *C. coluteoides* had the highest inhibition effects against all of tested gram positive bacteria. Also, *Bacillus cereus* was the most sensitive bacteria with inhibition zone of 18 mm. In antioxidant activity assay, different extracts of *C. coluteoides* exhibited various antioxidant activities due to the physical and chemical properties of solvent. In a way that, methanol extract of leaves showed the highest DPPH radical scavenging activity at various concentrations ranged from 5-160 $mg.ml^{-1}$. Among all tested extracts, the highest level of phenolics was observed in the methanol extract of flower.

Discussion and Conclusion: this study demonstrates that different extracts from various parts of *C. coluteoides* are different in their properties, so a proper solvent would be used to extract maximum amounts of antioxidant and antibacterial components from a typical plant material.

Keywords: *Cleome coluteoides*, Antibacterial, Antifungal, Antioxidant.

Cleome coluteoides Boiss Antibakteriyel, Antifungal ve Antioksidan Aktiviteleri: Yaprak, Çiçek ve Kök Ekstrelerinin In vitro Karşılaştırmalı Çalışması

ÖZET

Amaç: *Cleome coluteoides*, Capparidaceae familyasına aittir ve uzun süredir geleneksel tıp kullanılmaktadır. Araştırmamızın amacı *C.coluteoides*'in antioksidan, antibakteriyal ve antifungal aktivitelerini ölçmektir.

Gereç ve Yöntemler: Bitkinin farklı kısımları çeşitli solvanlarla (Etil asetat, metanol ve diklorometan) ile ekstre edildi. Antibakteriyal ve antifungal aktiviteler, well diffusion. Antioksidan aktiviteler ise DPPH ve FRAP yöntemleri ile tarandı.

Bulgular: Sonuçlar, gram negatif bakteri ve mantarların yukarıda bahsi geçen çeşitli bitki ekstrelerine karşı dayanıklı olduğunu gösterdi. *C. coluteoides*'in çiçek ekstresi, test edilen gram pozitif bakterilerin tümüne karşı en yüksek inhibitör etkiye sahipti. Ayrıca, *Bacillus cereus*, 18 mm inhibitör zonuyla en hassas bakteridir. Antioksidan aktivite analizinde, farklı *C. coluteoides* ekstreleri, solvanların fiziksel ve kimyasal özelliklerinden dolayı çeşitli antioksidan aktiviteler göstermişlerdir. Bir şekilde, yaprakların metanol ekstresi, 5-160 $mg.ml^{-1}$ arasında değişen konsantrasyonlarda en yüksek DPPH serbest radikal süpürücü aktivite göstermiştir. Test edilen tüm ekstreler arasında en yüksek phenolic seviyesi çiçeklerin metanol ekstresinde gözlendi.

TARTIŞMA ve SONUÇ: Bu çalışma, *C. coluteoides*'in çeşitli kısımlarının özelliklerinin farklı özelliklere sahip olduğunu göstermektedir, bu nedenle bir bitki materyalinin optimize edilmiş antioksidan ve antibakteriyel aktiviteleri için uygun bir çözücü bulmak hayati öneme sahiptir.

Anahtar Kelimeler: *Cleome coluteoides*, Antibakteriyel, Antifungal, Antioksidan.

Introduction

Free radicals are unstable and highly reactive atoms/groups with unpaired electrons, they can cause membrane damage, heart complications, aging and cancer; antioxidants can be used to protect damages caused by free radicals.[1] Natural antioxidants can efficiently improve the stability of drugs, foods, and nutrients and increase anti-inflammatory, anti-allergic, and anti-cancer potential of human body, on the other hand, plants with high phenolic contents are a good source of powerful antioxidants.[2] One of the most serious public health problems is microbial resistance to antibiotics, especially in developing countries that infectious diseases are a major cause of human mortality.[3] Thus, there is a great interest to find new compounds derived from medicinal plants. Recent studies have brought to light that organic herbs contain secondary metabolites which make them as good candidates for traditional or native remedies especially as antimicrobial and antifungal agents.[4] Recently, investigations have proved that pathogenic microbes can be controlled via medicinal plants based drugs.[5] Accordingly, it is essential to examine different medicinal plants extracts and constituents for their antibacterial activity.

The genus *Cleome L.* that belongs to Capparidaceae family is a large genus with 200 species worldwide. This genus has been used in folk medicine for a long time because of its ethnomedicinal properties including anthelmintic, carminative, anticonvulsant, antidiarhhoeal, antimicrobial, and wound healing effects.[6] *C. arabica L*, *C. viscosa* Linn, *C. droserifolia* (Forssk.), *C. enrichment, C. rutidosperma DC*, *C. gynandra* L are some species in this genus that have been used as a traditional medicine in treatment of scabies, inflammation, rheumatic pains, blood problems, uterine complaints, malaria, counteract diabetic hyperglycemia, treat paralysis, anthelmintic problems , epilepsy, convulsions, spasm, pain and skin disease and possess antipyretic and antidiarrhoeal properties.[7-11] According to the mentioned traditional applications, the different parts of *Cleome* genus like leaves, roots, and seeds are used as stimulant, antiscorbutic, anthelmintic, rubifacient, vesicant and carminative factors.[12] Phytochemical screening studies of *Cleome* genus have been found phenolic compounds, alkaloids, terpenoids, flavonoids, Fatty acids, coumarino-lignan, rich source of nutrients, especially vitamins A and C, protein, gallotannins, saponins, iridoid, hexacosanol and kaempferol.[7, 13-17] Therefore, finding natural antibacterial, antifungual and antioxdant agents with similar therapeutic effect could be a promising approach, which is safer and healthier.[18] To date, the chemical composition and pharmacological effects of various species of the genus *Cleome* have been reported, however to our knowledge the chemical components and extracts related activities of *C. coluteoides* have not

been shown. This study was focused on antioxidant, antibacterial and antifungal activities of various extracts of *C. coluteoides.*

Materials and Methods

Folin-Ciocalteau reagent (F9252, Sigma–Aldrich), Gallic acid (91215, Fluka), Aluminum Chloride (563919, anhydrous powder,99.999%, Sigma–Aldrich), 2,2-diphenyl-1-picrylhydrazyl (257621, Sigma–Aldrich), 2,4,6-tripyridyl-s-triazine (TPTZ) (T1253 for spectrophotometric, ≥98%, Sigma), Butylated Hydroxy Toluene (BHT) (W218405≤99, Sigma–Aldrich), Na_2CO_3 (451614, anhydrous powder, 99.999%, Sigma–Aldrich), $FeSO_4.7H_2O$ (21542, ≥99.0%, Sigma–Aldrich).

The stems, flowers and leaves of *C. coluteoides* were collected from Roein region from the North Khorasan Province of Iran at June 2017. The voucher specimen (MP.1247) was authenticated by botanist and it has been deposited at the Herbarium of natural products and medicinal plants research center in North Khorasan University of medical sciences. Bacterial and fungal strains were procured from Pasteur Institute of Iran.

Extracts preparation

Powdered stems, flowers and leaves of *C. coluteoides* were separately extracted with various solvents such as ethyl acetate, methanol and dichloromethane using maceration method at room temperature for 24 h. In the following, they were filtered through a paper filter and the solvent was evaporated under a vacuum at 45°C, to yield crude extracts.

Microorganisms

The microorganisms used for the antimicrobial activities screening were *Escherichia coli* (ATCC 8739), *Bacillus cereus* (PTCC 1247) and *Staphylococcus aureus* (ATCC 6538). Fungal strains applied for antifungal tests were *Fusarium solani* (PTCC 5284), and *Candida albicans* (ATCC 10231). In this study, antimicrobial activity was assayed by disk and well diffusion methods (CLSI 2012 standard).

Well diffusion method

Wells with 6 mm diameter were punched into the agar plates with appropriate media (Mueller Hinton agar (MHA) for bacterial and Soybean Casein Agar (SCA or Tryptic Soy Agar) for

fungal strains. A density of 1.5×10^8 microorganism cells had been spread on the plates surface. Next, 50 µl of plant extracts (100 $mg.ml^{-1}$) were added into the wells. Incubation length for bacteria was 16-20 h at 37 °C and incubation length for fungi was 36-48 h at 32 °C and then the zones of inhibition (mm) were measured.[19]

Disk diffusion method

The discs contained plant extracts were dissolved in dimethyl sulfoxide (DMSO) to give 100 $mg.ml^{-1}$ concentration with 6 mm diameter were placed on the plates with 100 mm diameters containing Mueller Hinton agar (MHA) and Soybean Casein Agar (SCA) for bacterial and fungal strains with 1.5×10^8 microorganism cells. Incubation length for bacteria was 16-20 h at 37 °C and incubation length for fungi was 36-48 h at 32 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition surrounding the discs. Gentamycin and Nystatin were positive control and DMSO was used as a negative control. [20]

Evaluation of antioxidant activity

Free radical scavenging activity assay

The free radical scavenging activity was assessed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH). To do so, 100 µl of each extract (5-160 mg/ml) incubated with 200 µl DPPH (300 µM) at 37 °C for 30 min in a 96-well microplate. After that, absorption was measured at 490 nm using a ELISA microplate reader (BioTek). MeOH and butylated hydroxytoluene (BHT) were respectively used as blank and positive controls. Inhibition of free radical DPPH was calculated as follows:

$$\% \text{ Inhibition} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where A_{blank} was the absorbance of the blank group, and A_{sample} was the absorbance of each extract.[21]

Ferric reducing antioxidant power (FRAP) assay

Ferrous Sulphate (10–100 mM $FeSO_4 \cdot 7H_2O$) was used as a standard. 100 μL of each extract (50 mg/ml) was added to FRAP reagent (3 ml) and then was incubated at 37^0C for 10 min in a water bath. After incubation, the absorbance was measured at 600 nm by using ELISA microplate reader. All tests were performed in triplicates. The results were presented as means ± SD. FRAP values were expressed as mmol Fe (II) per gram of extract. [22]

Determination of total phenolic content (TPC)

10 µl of each extract was added to 100 µL of Folin-Ciocalteu's phenol reagent (0.2 N) in 96-well plates. After 3 min, 90 µL of saturated sodium carbonate solution was added to the mixture and incubated for 1 h at room temperature. The absorbance of samples was measured at 630 nm. The total phenolic content was calculated by standard curve using gallic acid (concentration range 3.25 to 500 mg·mL^{-1}). Results were expressed as microgram gallic acid equivalent per milligram dry weight (µg GAE/mg DW).[21, 22]

Statistical analysis

Data were expressed as mean ± SD for at least three independent determinations in triplicate for each experimental point. IC_{50} values in DPPH test were calculated using GraphPad Prism version 5.01 software.

Results

As expected, different extracts from various parts of *C. coluteoides* represented different amounts of extractable yields. Extracts expressed as DEL: dichloromethane extract of leaves; MEL: methanol extract of leaves, EEL: ethylacetate extract of leaves, DEF: dichloromethane extract of flower ; MEF: methanol extract of flower , EEF: ethylacetate extract of flower DES: dichloromethane extract of stem, MES: methanol extract of stem, EES: ethylacetate extract of stem. The highest yield (9.63%) was obtained by MEF and the lowest ones were related to DES and EES. Altogether, methanol solvent system could be more efficient to get more yields from various parts of the plant. Dried extracts (ethyl acetate, methanol and dichloromethane extracts) of stems, flowers and leaves were tested for antibacterial and antifungal activities by disc/well diffusion methods and their results have been shown in Table 1. Based, diameter of inhibitory zones for gram negative bacteria and fungus were 6.0 mm in both methods and all of microorganisms were resistant to various plant extracts. So, the results of well diffusion method were similar to disc diffusion method. Flowers extract of *C. coluteoides* had the highest inhibition effects against all of the tested microorganisms. However, its activity was markedly lower than gentamicin in bacteria. *B. cereus* was more sensitive bacteria with inhibition zone of 18 mm in disc diffusion method.

Free radical scavenging activities of various extracts are presented in Table 2. It should be mentioned that a lower IC_{50} value indicates a higher antioxidant activity. The MEL exhibited remarkable antioxidant activities tested by DPPH radical scavenging activity (19.54±0.21 mg/m1). The IC_{50} of all plant parts was higher than standard BHT which was 0.15 mg/ml. Also,

DPPH radical scavenging activity of test samples rapidly increased in a dose dependent manner from 5 to 160 mg/ml (Figure 2). According to the findings, dichloromethane extracts possessed the lowest antioxidant activity. Contents of total phenolic compounds were expressed in mg EAG/g and the results are displayed in Table 3. Total phenolic compounds extracted from samples significantly varied to each other. Amongst, methanol extracts show higher phenolic contents respectively followed by ethyl acetate and dichloromethane extracts.

Discussion

Screening plant parts for antioxidant and antibacterial activities were performed by extracting different parts of *C. coluteoides* including leaves, flowers and stems with various types of solvent (methanol, dichloromethane and ethylacetate). Variation in the extract yields of the plant may be due to the various chemical natures of the compounds available in the leaves, barks and seeds; also the extraction yields were affected by the polarity, solubility, concentration, pH, temperature and nature of the extraction solvents.[23] Hence, a proper solvent system has to be used for extraction of maximum amounts of potent antioxidant components from typical plant materials.[24] On the one hand; medicinal plants produce secondary metabolites that are responsible for their therapeutic properties. On the other hand, quality and quantity of these molecules and conversely their activities are affected by environmental factors like geographical locations and origin of plants.[25] In the present study, the extracts of *C. coluteoides* were found to be inactive against tested bacterial and fungal strains. In return, gram positive bacterial strains were dedicated better resistant to the extracts. Antimicrobial functions of medicinal plants are reported from several parts of the world. So that, the World Health Organization estimates that medicinal plants or their active constituents could be applied in traditional.[26] It has been evidenced that methanol extract of *C. viscosa* has exhibited the highest and significant antibacterial activity against seven bacterial strains.[27] Subsequently, ethanolic extract of *C. ciliata* had inhibitory effects against *S. aureus*, *P.aeruginosa* , the tannin fraction was active only against *S. aureus*.[28] Antimicrobial test of methanolic extract of *C. viscosa* has displayed moderate sensitivity to the gram positive and gram negative bacteria. The highest zone of inhibition (16.34 mm) was recorded against *Shigella sonnie*.[15] Crude extracts *of C. viscosa* have presented antibacterial potency against *P. aeruginosa*.[5] Other researchers reported the presence of alkaloids and tannins in *C. viscosa* and they are associated with antibacterial activity.[5]

Antioxidant activity of extracts was evaluated by two methods, DPPH and FRAP tests. The DPPH assesses the potential of antioxidants to naturalize free radicals through reduction mechanisms, which discolourate stable free radical DPPH of violet color to yellow one.[29] It is well known that DPPH scavengers have a wide range of biological functions such as lipid peroxidation inhibitory action and radioprotective.[30] There is line of evidence that show antioxidant and pharmacological properties of plants are usually associated with presence of phenolic compounds and as electron donor agents.[31] In this study, strong antioxidant activity of flowers extract probably due to the phenolic contents.[21] Similarly, butanol fractions of *C. gynandra,* have demonstrated remarkable anti-FRAP, anti-ABTS, and anti-DPPH activities. Moreover, the best phenolic content of *C. gynandra* was obtained with n-butanol fraction.[16] Phenolic and antioxidant compounds can form soluble and stable complex of iron that can easily be excreted from the body.[32] In Table 2, ferrous chelating activities (known as an important antioxidant mechanism) of extracts were summarized. According to the results, methanol extract had greater chelating action which was due to its higher phenol contents. As indicated in Table 3, the total phenolic contents of various extracts of *C. coluteoides* were ranged from 5.24±0.57 to 24.58±0.35 mg GAE/100 g DW. The highest phenolic content was found in the MEF (24.58±0.35 g GAE/g DW). Furthermore, phenolic compounds were found in the following order; MEF>MEL>EEF>EEL>DEF>MES>EES>DEL>DES. Collectively, DPPH findings were in agreement with the phenolic contents in each sample. In keeping with our results, plant phenolics act as reducing agents and antioxidants via hydrogen-donating property of their hydroxyl groups.[33] Hence, antioxidant activity observed in this study could be explained by polyphenol contents. Thus, it can be concluded that methanol solvent was the best solvent for the extraction of antioxidant activity of *C. coluteoides*. Our phenolic results of different plant parts were in agreement with previous investigation in which it was reported greater amounts of total phenolics in the leaves than the stems parts.[34] A larger amount of phenolic and flavonoids in plants leaves might be the result of photosynthesis in this section.[35] Consistently, the highest amounts of total phenols in stems extract of *C. gynandra* L. have been determined in its aqueous extract.[36] The varied scavenging activity in different parts of the plants could be attributed to the presence of bioactive compounds such as phenolics, flavonoids and tannins.[37] Previously, a strong correlation has been observed between radical scavenging power of plant extract with total phenolics and flavonoids contents.[24] The present results provided evidence that most extracts with high contents of phenolics exhibited greater potency to scavenge free radicals.

Nevertheless, some extracts with less amounts of phenolics depicted appreciable activity suggesting the presence of other secondary metabolites (carotenoids, volatile oils and vitamins) may also contribute to scavenging capacity.[38] Accordingly, in the current study MES had fewer amounts of phenolics than flowers one, but it exhibited greater antioxidant activity.[39,40] Totally, our antioxidant and antibacterial activity results are consistent with other *Cleome* species related studies which was mentioned earlier.[16]

Conclusion

In conclusion, this study demonstrates that different extracts from various parts of *C. coluteoides* are different in their antioxidant and antimicrobial effects. The results indicate that the MEL possessed the highest antioxidant properties and EEF had the highest antimicrobial activities, which were probably correlated with total phenolics. Phytochemicals analysis of *C. coluteoides* should be carried out to characterize the antioxidant and antibacterial agents. This work demonstrates that extracting various parts of the plants with different solvents have variable antioxidant and antibacterial activities. To our knowledge, this is the first report revealing the antioxidant and antibacterial value of *C. coluteoides.*

Acknowledgements

This study was financially supported by North Khorasan University of Medical Sciences, Bojnurd, Iran.

Conflict of Interest

No conflict of interest was declared by the authors.

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Table 1: Antifungal and antibacterial activities of dried extracts of *C. coluteoides* in disc and well diffusion method.

Sample	**Microorganism[a]**						
	B. cereus		***S.aureus***		***E. coli***	***C. albicans***	***F.solani***
	Disc	**Well**	**Disc**	**Well**	**Disc& Well**	**Disc& Well**	**Disc& Well**
MES [b]	8.3±0.57	9.0±0.24	9.3±0.63	10.3±0.12	- [c]	-	-
MEF	10.0±0.14	10.0±0.28	10.3±0. 74	11.3±0. 37	-	-	-
MEL	14.3±0.24	10.0±0.47	12.0±0.52	6.0±0.35	-	-	-
EEF	18.0±0.89	15.0±0.16	6.0±0.41	6.0±0.21	-	-	-
EEL	15.3±0.43	11.0±0.38	11.3±0.14	11.6±0.26	-	-	-
DEF	15.0±0.16	11.6±0.37	6.0±0.21	6.0±0.14	-	-	-
DEL	16.3±0.35	11.3±0.41	6.0±0.16	10.0±0.12	-	-	-
Gentamycin	30.3±0.19	32.3±1.15	31.3±0.21	40.0±0.23	29.6±0.15	-	-
Nystatin	-	-	-	-	-	25.0±0.25	26±0.14

[a] Antibacterial and antifungal activities of extract were evaluated by presence or absence of inhibition zone based on zone diameters (mm).

[b] DEL: dichloromethane extract of leaves; MEL: methanol extract of leaves, EEL: ethylacetate extract of leaves, DEF: dichloromethane extract of flower ; MEF: methanol extract of flower , EEF: ethylacetate

extract of flower DES: dichloromethane extract of stem, MES: methanol extract of stem, EES: ethylacetate extract of stem

[c] non-effective

Table 2. Free radical scavenging activity and ferric-reducing antioxidant power (FRAP) of different extracts from various parts of *C. coluteoides*.[a]

Sample	FRAP(mmol Fe^{2+}/g extract)	DPPH IC_{50} (mg/ml)
MES[b]	-[c]	49.35±0.25
MEF	68.24±0.12	24.02±2.02
MEL	90.98±0.12	19.54±2.41
EES	59.44±0.18	58.45±3.48
EEF	81.03±0.19	157.25±1.49
EEL	-	159.33±1.64
DES	61.68±0.45	99.05±5.17
DEF	-	424.02±0.25
DEL	-	169.90±2.66
BHT[d]	14.32±0.58	0.40±0.03

[a]Data are expressed as mean ± SD (standard deviation), (n=3).
[b]DEL: dichloromethane extract of leaves; MEL: methanol extract of leaves, EEL: ethylacetate extract of leaves, DEF: dichloromethane extract of flower ; MEF: methanol extract of flower , EEF: ethylacetate extract of flower DES: dichloromethane extract of stem, MES: methanol extract of stem, EES: ethylacetate extract of stem
[c]non-effective
[d]positive control

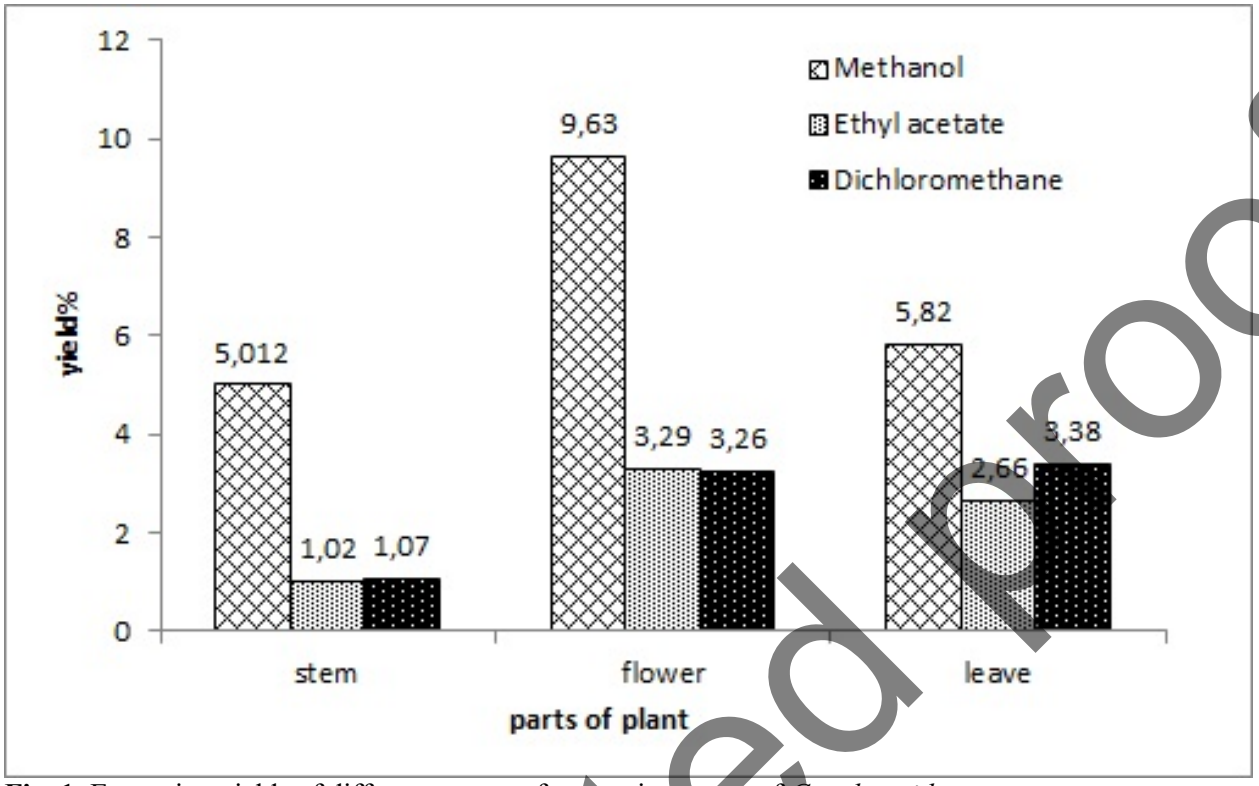

Fig. 1: Extraction yields of different extracts from various parts of *C. coluteoides.*

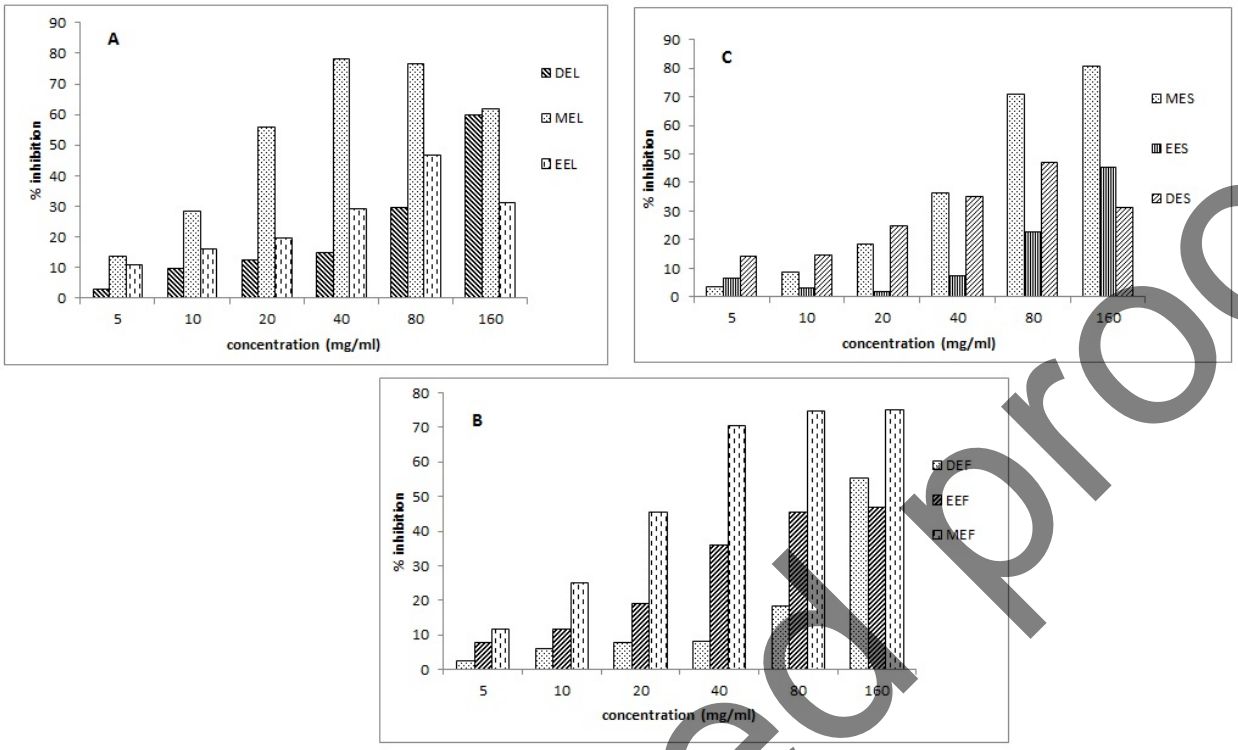

Fig. 2: Radical scavenging activities of various extracts from different parts of *C. coluteoides*; A: leaves parts; DEL: dichloromethane extract of leaves; MEL: methanol extract of leaves, EEL: ethyl acetate extract of leaves. B: flower parts; DEF: dichloromethane extract of flower ; MEF: methanol extract of flower , EEF: ethyl acetate extract of flower, C: stem parts of the plant; DES: dichloromethane extract of stem, MES: methanol extract of stem, EES: ethyl acetate extract of stem.