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Preliminary phytochemical screening, analysis of phenolic compounds and antioxidant activity of *Genista cephalantha* Spach. (Fabaceae)

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Abstract

This study was designed to estabilish a preliminary phytochemical screening, evaluate the phenolic and flavonoid content according to the Folin-Ciocalteu procedure, and aluminum chloride method respectively and to determine qualitatively, using HPLC-UV method, the most important products present in ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) extracts of the aerial parts of *Genista cephalantha* Spach. from east Algeria. The antioxidant activity of these extracts was spectrophotometrically tested by measuring their ability to scavenge a stable DPPH free radical and by β-Carotene/linoleic acid bleaching assay. Evaluated extracts showed a good activity in both antioxidant system assays.

Keywords: Phenolic compounds; Flavonoids; HPLC-DAD-UV; Antioxidant activity; *Genista cephalantha;* Fabaceae.

Introduction

Several studies have demonstrated the potential of plant products as antioxidants against various diseases induced by free radicals; so, these plants could constitute a major source of new chemical compounds which may be characterized by medicinal and biological properties. It has been determined that the antioxidant effect of plants is essentially attributed to phenolic compounds [1]. These antioxidant compounds could possess the capacity to protect the cellular organelles from damage caused by free radicals induced oxidative stress [2].

The genus Genista (Fabaceae), consisting in about 100 species, is predominantly distributed in the Mediterranean area and in Western Asia [3]. This genus is present in Algeria with 25 species and sub-species from which 11 are endemic [4].

Genista is known to contain flavonoids and isoflavonoids [5-6] as well as quinolizidine alkaloids [7-8]. As a part of our continuing studies on *Genista* species (Fabaceae), occurring in Algeria [9-14], we have examined *Genista cephalantha* Spach. (Fabaceae, subfamily Papilionoideae, tribe Genisteae), which is an endemic shrub of the east of Algeria growing in arid areas and flowering from May to June [3].

Genista species are reported to be medicinal plants [15-17]. In Algeria, many species from *Genista* genus have been used for traditional healing in the east region, but unfortunately, no report about *Genista cephalantha* Spach. has been mentioned.

In the present paper, we report the phytochemical screening and the evaluation of the antioxidant activity of the EtOAc and *n*-BuOH soluble parts of the aqueous ethanol extract from the aerial parts of *G. cephalantha* Spach. on which no reports on the evaluation of any biological activity nor on the isolation of any secondary metabolites are available to date.

Vegetal material

Aerial parts of *G. cephalantha* Spach. was collected during the flowering phase in June 2010 from the area of M'Sila in the Eastern Algeria, and was authenticated by Dr. D. Sarri (Biology department, University of M'Sila, Algeria). A voucher specimen (FGC10/06/10) has been deposited in the Herbarium of the VARENBIOMOL unit research, University of Constantine1.

Experimental methods

Phytochemical screening

Flowers and stems were separated and subjected to qualitative tests in order to characterize several chemical groups using standard procedures [18-19]. The symbols ++, + and – denote present, moderately present and absent respectively.

Extraction procedure

The air-dried aerial parts of *G. cephalantha* Spach. (2700 g) were macerated with EtOH: H_2O (70:30 v/v) three times for 72 hours. After filtration, the crude extract was concentrated and diluted with 900 ml H_2O. The obtained solution was extracted with petroleum ether to remove chlorophylls. The remaining aqueous solution was extracted successively with CH_2Cl_2, EtOAc, and *n*-BuOH, giving CH_2Cl_2 (3.52 g), EtOAc (6.54 g) and *n*-BuOH (33.73 g) extracts.

Estimation of secondary metabolites

Total phenolic content (TPC) quantification

The total phenolic content of EtOAc and *n*-BuOH extracts of *G. cephalantha* Spach. was spectrophotometrically determined according to the Folin-Ciocalteu method [20] using Gallic acid as a standard (the concentration range was 5 to 250 µg/ml). The total phenolic content was expressed as GAE in mg/g of extract.

Total flavonoid content (TFC) quantification

The total flavonoid content of EtOAc and *n*-BuOH extracts was expected according to the aluminum chloride colorimetric method [21]. Quercetin was chosen as a standard (the concentration range was 5 to 250 µg/ml) and the total flavonoid content was expressed as QE in mg/g of extract.

Characterization of phenolic and flavonoid compounds by HPLC

The HPLC/UV(DAD) analysis of phenolic and flavonoid compounds in both extracts was carried out using a HPLC system (Beckman Coulter System Gold) consisting in a model LC-508 autosampler, with a system Gold solvent delivery module 126 and a diode array detector Beckman system Gold 168, a column oven and Data Station for data acquisition and analysis. The column LICHROSPHER 100-5 RP-18 (250x4.6mm, 5µm) Macherey-Nagel was used. The column was thermostated at 18 C. Samples of 20 µl (0.32 mg/ml EtOAc extract and 0.16 mg/ml *n*-BuOH extract) were injected. Water (0.11% phosphoric acid conc.) was used as solvent A. Acetonitrile (ACN) was used as solvent B. The flow rate was 1.0 ml/min. Following gradient was used for elution: 5 % of B at 0 min, 30 % of B at 40 min, 90 % of B at 70 min and 5 % of B at 90 min. A wavelength of 340 nm was used for the detection of flavonoids.

The tentative identification of the class of each compound was carried out comparing the retention time and UV spectra of the peaks with the literature data.

DPPH radical scavenging activity

The free radical scavenging activity of both extracts was determined using DPPH. DPPH solution (0.2 mM) was prepared in 95% methanol. Each extract was mixed with methanol to prepare the solution (0.5 mg/ml). Freshly prepared 2 ml of DPPH solution was added to 2 ml of various concentrations (10- 200 µg/ml) of each extract. After 30 min, absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Ascorbic acid was used as a reference compound [22]. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with the extract) using the following formula:

Radical scavenging activity (%) = $(A_{control} - A_{sample}) / A_{control}$ 100,

where: $A_{control}$ is the absorbance of blank, A_{sample} is the absorbance of the sample.

β-Carotene/linoleic acid bleaching assay

The antioxidant activity was determined by the method described by Miraliakbari and Shahidi [23] with slight modifications. A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 ml chloroform, 25 µl of linoleic acid and 200 mg Tween 40. The chloroform was evaporated under vacuum and 100 ml of oxygenated distilled water were then added to the residue. 350 µl of each sample solution (2 g/l) prepared in DMSO were added to 2.5 ml of the above mixture in test tubes which were incubated at 50 C for 24 h. BHT and trolox were used as a positive control. The absorbencies were measured at 490 nm each three hours during 24 h. Antioxidant activities (inhibition percentage) of the samples were calculated using the following equation:

Inhibtion % = (Absorbance of β-carotene at 24 h /Initial absorbance of β-carotene) 100.

Statistical analysis

All assays were carried in triplicates and results expressed as means ± standard deviation. Statistical comparisons were done with Student's test. Differences were considered to be highly significant at $P< 0.01$ and significant at $P< 0.05$.

Results and discussion

Phytochemical screening

Qualitative phytochemical screening revealed that flowers and stems contained some phytoconstituents in various portions. Alkaloids, flavonoids and saponins are strongly present in both organs (Table 1). These bioactive components are naturally occurring in most *Genista* species and known to possess interesting biological activities [24-27].

Table 1: Phytochemical screening from *Genista cephalantha* (Fabaceae)

Chemical groups	Flowers	Stems
Terpenes	+	+
Sterols	-	-
Triterpenes	-	-
Saponins	++	++
Coumarins	+	+
Flavonoids	++	++
Leucoanthocyans	-	-
Anthocyans	-	-
Quinones	+	+
Tannins	++	+
Alkaloids	++	++

Total phenolic and flavonoid contents

Several studies have shown that a diet rich in fruit and vegetables has an important role in reducing the incidence of diseases. Some of these preventive actions have been related to the presence of bioactive substances such as polyphenols. Among phenolic compounds, flavonoids belong to a large group widely distributed in edible plants. The global interest in flavonoids is due to their antioxidant properties and bioactivity [28-29]. TPC and TFC of EtOAc and *n*-BuOH extracts of *G.* cephalantha are given in figure1. The TPC level was higher in the n-BuOH (156.86±42.98 mgGAE/g extract) compared to the EtOAc (124.64±20.22 mgGAE/g extract). The n-BuOH fraction was also richer in flavonoids (125.03±18.42 mgQE/g extract) (79.71 %) than EtOAc fraction (65.83±4.35mgQE/g extract) (52.82 %).

Characterization of phenolic and flavonoid compounds by HPLC

Both EtOAc and *n*-BuOH extracts of the aerial parts of *G. cephalantha* Spach. were analysed by HPLC-UV method. The resulting chromatogram obtained at 340 nm and the UV spectra of selected peaks are shown in figure 2 (2a and 2b). The figure 2a showed that the EtOAc extract contained several main peaks which had intense absorption at the selected λ (340 nm). According to their UV spectra profiles, these compounds are phenolic and flavonic types, especially, the compound corresponding to the peak 20 seems to be the main important. This peak (t_R= 52.19 mn) presented spectral characteristics of an isoflavone skeleton with UV λ_{max} at 270, 300 and 328sh nm.

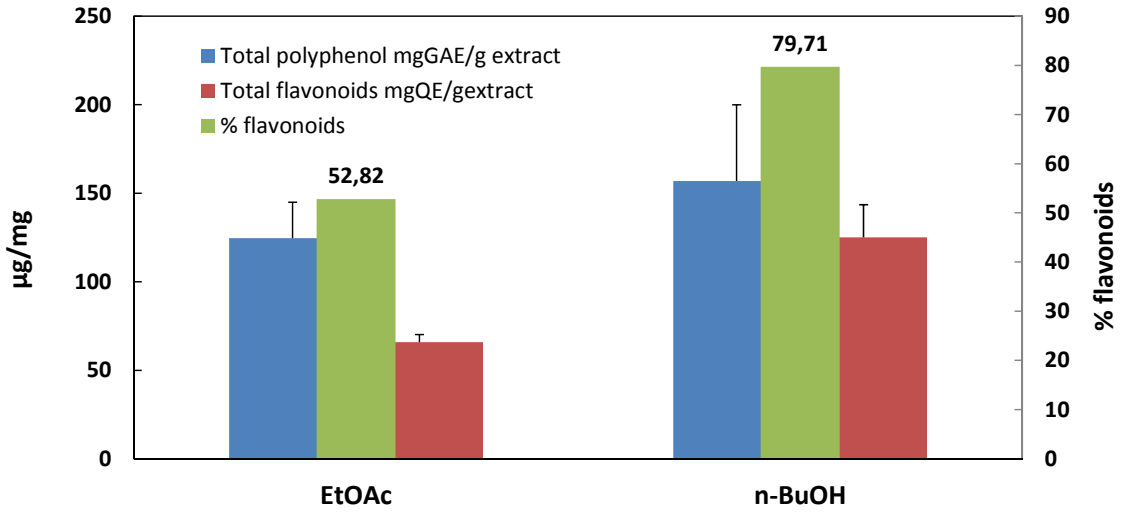


Figure1: TPC (µgGAE/mg extract), TFC (µgQE/mg extract), and flavonoids percentage (%) *G. cephalantha* extracts. Each value represents a mean ±SD (n=3), p<0.05).

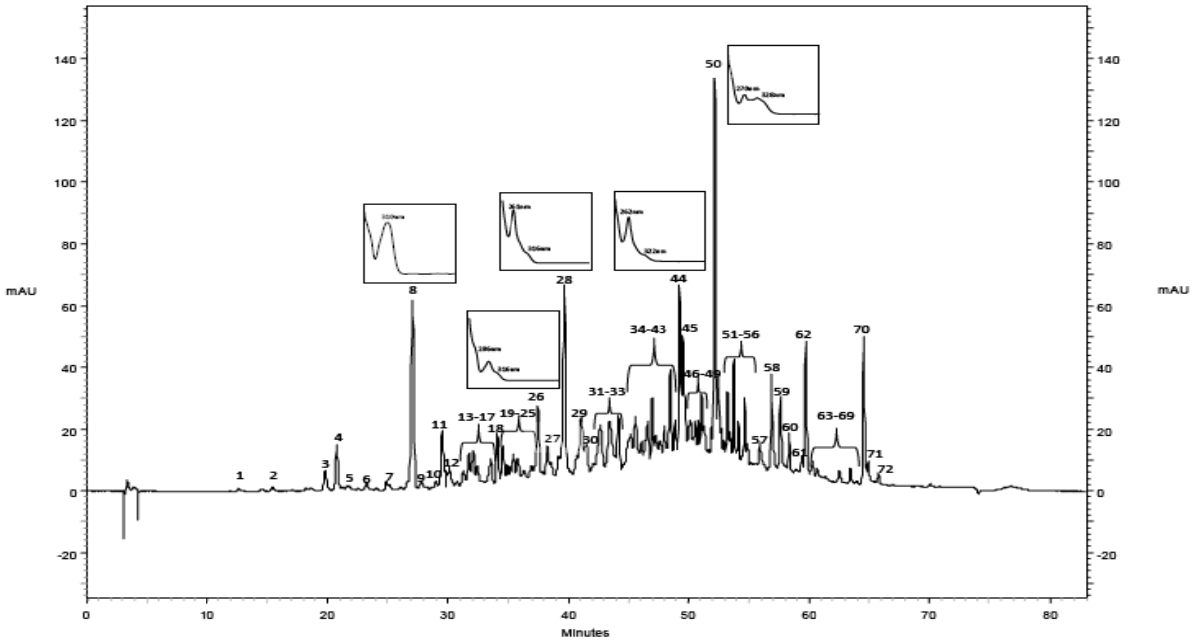


Figure 2a: HPLC chromatogram of the EtOAC extract from the aerial parts of *G. cephalantha* Spach. detected at 340 nm. Peak numbers were consistent with those shown in table 3a

However, on the chromatogram of the *n*-BuOH, the peak 31(t_R= 34.21 nm) is strongly prominent (Figure 2b). This compound presented spectral characteristics of a flavonol skeleton with UV λmax at 257, 310 and 355 nm. Generally, most of the constituents of the EtOAc extract had similar UV spectra with a maximum of absorbance of band II varying between 254 and 268 nm and a maximum of band I as shoulder varying between 315 and 325 nm indicating isoflavonoid like character of the compounds [30]. Isoflavones may be distinguished from flavones and flavonols by their UV spectra which typically exhibit intense band II absorption with a shoulder peak representing band I [31]. These results indicate clearly the richness of this species in flavones, flavonols and isoflavones compounds.

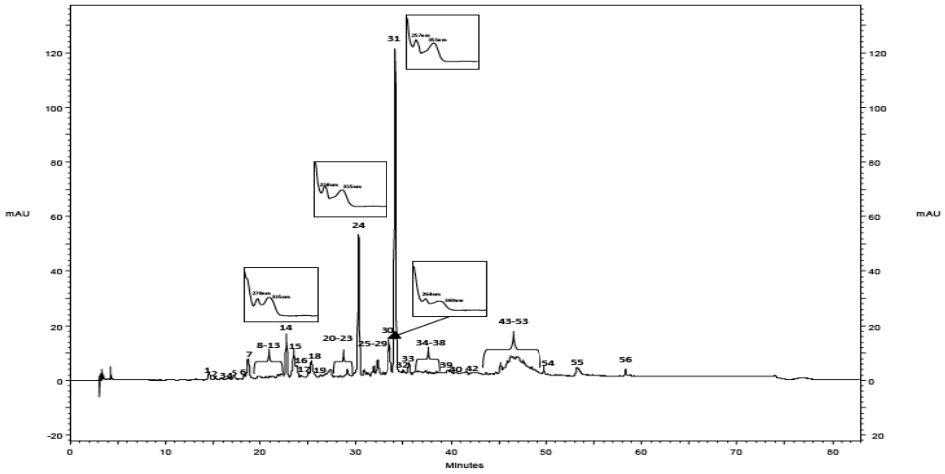


Figure 2b: HPLC chromatogram of the *n*-BuOH extract from the aerial parts of *G. cephalantha* Spach. detected at 340 nm. Peak numbers were consistent with those shown in table 3b.

As shown in table 3 (3a and 3b), tentative identifications have been made by examination and comparison of their retention time (t_R) and UV spectra with literature data.

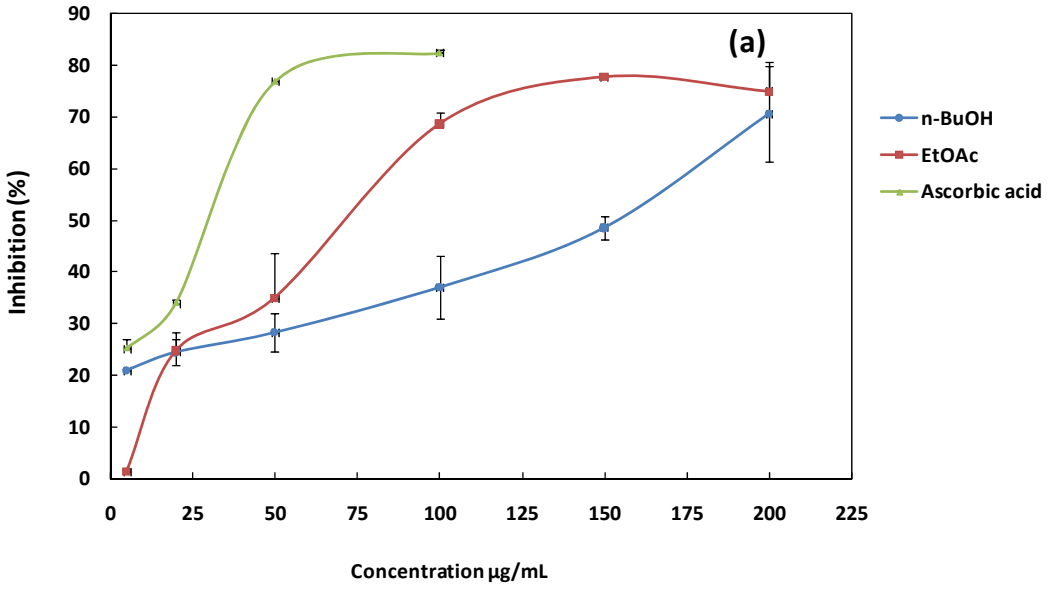
Table 3: Tentative identification of phenolic and flavonoid contents in ethyl acetate extract and *n*-butanol from the aerial par of *G. cephalantha* Spach. by HPLC-DAD.

Table 3a : Ethyl acetate extract				Table 3b: *n*-butanol extract			
Peak no	R_T (min)	λ_{max} (nm)	Tentative identification	Peak no	R_T (min)	λ_{max} (nm)	Tentative identification
1	12.71	257	GallicAcid	1	14.62	260	Gallic acid
2	15.42	262, 288	Phenol	7	18.76	240, 305, 325	Phenol
3	19.77	265, 298, 342	Phenol	14	22.76	270, 335	Isovitexin
4	20.83	300, 328	FerulicAcid	17	25.00	252, 280sh, 320sh	Isoflavonoid
8	27.10	310	Phenol	20	27.45	320	Phenol
14	31.72	287, 315 sh	Isoflavonoid	23	29.12	272, 340	vetexin
15	32.06	262, 315 sh	Isoflavonoid	24	30.30	258, 305, 355	isoquercetrin
28	39.66	261, 316 sh	Isoflavonoid	27	31.86	250, 300, 325	Flavonoid
31	42.70	262, 318 sh	Isoflavonoid	28	32.23	260, 325 sh	Isoflavonoid
33	44.17	268, 320 sh	Isoflavonoid	30	33.51	264, 340	Flavonoid
34	45.22	268, 320 sh	Isoflavonoid	31	34.21	257, 310, 355	Flavonoid
38	47.00	268, 320 sh	Isoflavonoid				
44	49.31	262, 322 sh	Isoflavonoid				
45	49.55	268, 322 sh	Isoflavonoid				

Antioxidant activity

DPPH radical scavenging activity

Hydrogen donating ability of extracts was measured by using DPPH assay and the results were expressed in percentage of inhibition of DPPH . Figure 3a shows the free radical scavenging ability of EtOAc and *n*-BuOH extracts. The EtOAc extract which was more effective than *n*-BuOH showed a gradual increase in DPPH radical scavenging activity in a dose dependent manner.



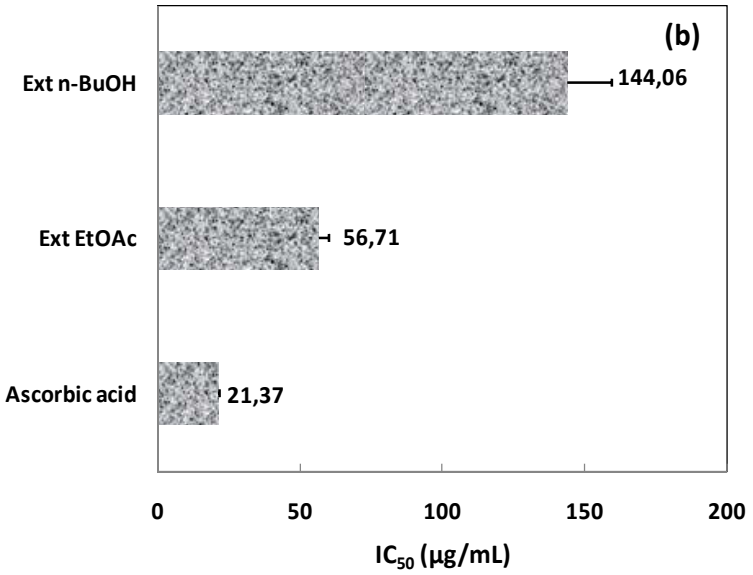
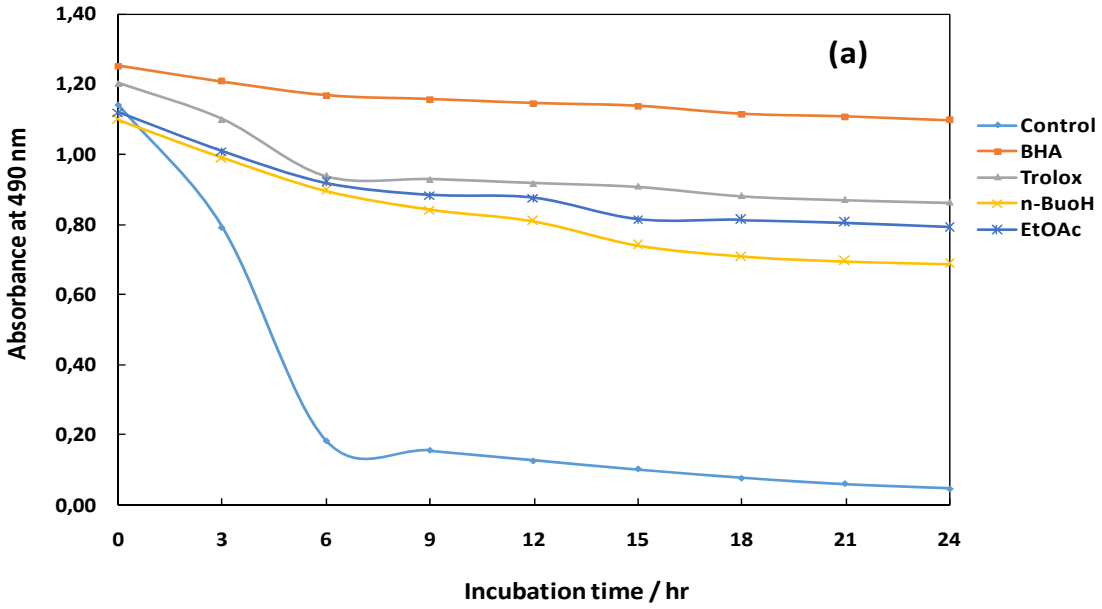


Figure 3: DPPH radical scavenging activities (a) and DPPH IC_{50} values (b) of *G. cephalantha* and standard. Each value represents a mean ± SD (n=3), P<0.05

The maximum inhibition value (77.78 ± 0.03 %) was observed at 150µg/ml, there after the curve reach a plateau. DPPH scavenging activity of n-BuOH extract was slightly lower than this of EtOAc extract, its curve reach 70.57 ± 9.25 % at 200µg/ml. Though TPC and TFC of n-BuOH extract was higher than those of EtOAc, its DPPH IC50 (144.06 ± 15.30 µg/ml) was significantly (P<0.05) lower than the IC50 of EtOAc (56.71 ± 3.45 µg/ml) (Figure 3b).

β-Carotene/linoleic acid bleaching assay

The oxidation of linoleic acid produces hydroperoxides derived free radicals which attack the chromophore of β-carotene resulting in bleaching of the reaction emulsion [32]. Figure 3a showed that there was a decrease in absorbance value due to the oxidation of β-carotene.



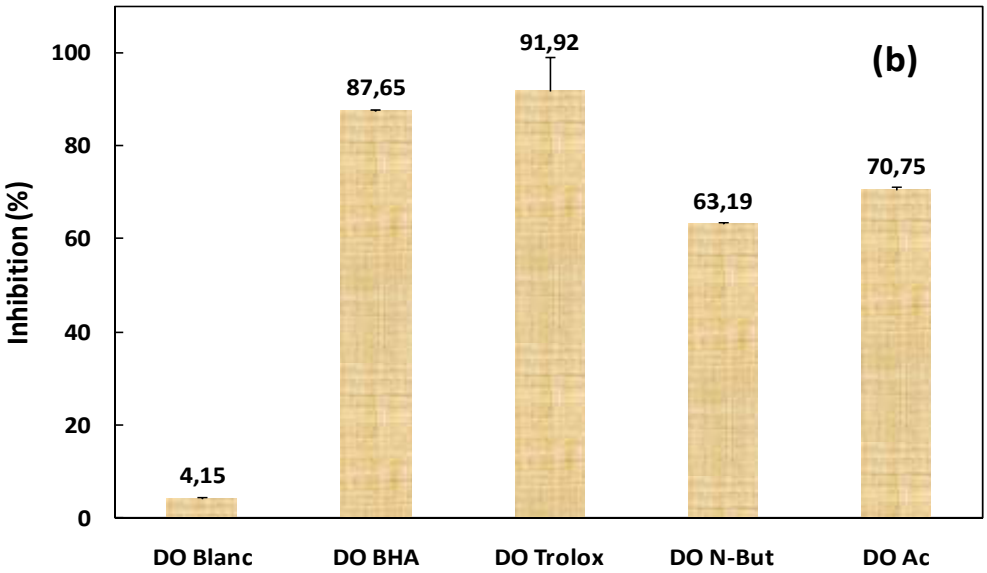


Figure 4: Effect of *G. cephalantha* antioxidant on oxidation of β-Carotene/linoleic acid (a) and inhibition percentage β-carotene bleaching (b).

At 1 mg/ml both extracts exhibited a good antioxidant activity (63-70 %) as compared to standards substances BHA and trolox (87-91 %) by protecting β-carotene from auto-oxidation, due to free radical chain generation from linoleic acid peroxidation. The high absorbance noticed from EtOAc extract indicated that EtOA extract possessed the high antioxidant power. The presence of varying amount of antioxidant compounds in both extracts can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radicals and other free radicals formed in the system [33]. Inhibition of lipid peroxidation by donating a hydrogen atom is the basis of the β-carotene bleaching assay. The abilities both extracts and standards to retard the lipid peroxidation were in order of Trolox (91.92 %) > BHA (87.65 %)> EtOAc (70.75 %)>*n*-BuOH (63.19 %) (Figure 4b).

Our phytochemical screening indicated that *n*-BuOH extract which had high TPC and TFC levels exhibited less antioxidant activity as compared to EtOAc extract which had less TPC and TFC levels indicating that the concentration of TPC and TFC levels are not the only factor revealed to the antioxidant activities. According to the HPLC analysis, both extracts afforded different polyphenol profile (Figure 2) such as flavones, flavonol, isoflavone and phenolic acid compounds, which were attributable to their antioxidant effects.

The presence of varying amount of polyphenol compounds in both extracts can reflect the different antioxidant behavior. The more effectiveness EtOAc soluble part stated its richness in phenolic acids (gallic acid, ferrulic acid), flavonoid, and the isoflavone which was the predominant compound would have an important contributing factor to the antioxidant power. The high antioxidant activity of EtOAc extract is probably attributed to the presence of more active substances (mainly the isoflavone) than those found in *n*-BuOH extract (vitexin). The possible synergism of the active compounds and their redox properties may be responsible for the antioxidant behavior.

Conclusion

The phytochemical screening of this investigation attested the presence of several secondary metabolites in *G. cephalantha.* The fractionation of MeOH extract into two different solvents (EtOAc and *n*-BuOH) resulted into an interesting distribution of TPC and TFC. Both *n*-BuOH and EtOAc extracts exhibited good antioxidant activities (DPPH scavinging, β-bleaching). The presence of more active substances in EtOAc extract than those found in *n*-BuOH extract reflects the different redox properties. Thus *G. cephalantha* could be candidate for a good source of antioxidant compounds.

Author's contribution

MR is supervisor of this study participated in the design conception of this study and involved in drafting the manuscript.
CK prepared the plant extracts and phytochemical studies.
ME contributed in carrying HPLC-DAD analysis
BO and SR collected the plant and preparing the plant extracts.
SD identified the plant material
AS and MA, have an equal contribution in carried antioxidant activity and statistical analysis, and helping in the drafting the manuscript.
BF and BS have general supervision of the research group.

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