World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

# ISOLATION, PARTIAL CHARACTERIZATION OF A DIHYDROFLAVONOL FROM SUDANESE *CISTANCHE PHELYPEA* STEMS AND BIOLOGICAL ACTIVITY OF THE ETHANOL EXTRACT

**Prof. Abdel Karim M.[1*], Nousiba M.[1] and Inas O.[2]**

[1]Sudan University of Science and Technology, Faculty of Science.
[2]University of Bahri, College of Applied and Industrial Chemistry.

***Corresponding Author: Prof. Abdel Karim M.**
Sudan University of Science and Technology, Faculty of Science.

**Article Received on 15/07/2019** **Article Revised on 05/08/2019** **Article Accepted on 25/08/2019**

## ABSTRACT

Phytochemical screening of *Cistanche phelypea* stems revealed the presence of flavonoids, tannins steroids, saponins, terpenes and alkaloids. A flavonoid - compound (I)- was isolated from ethanol extract by thin layer chromatography and its structure was partially characterized on the basis of its spectral data (IR, UV, NMR). The ethanol extract of *Cistanche phelypea* was screened for antimicrobial activity against five standard human pathogens : *Bacillus subtilis* (Gram((+ve), *Staphylococcus aureus* (Gram (+ve), *Pseudomonas aeroginosa* (Gram(–ve), *Escherichia coli*(Gram (–ve) and the fungal species *Candida albicans*.The ethanolic extract of *Cistanche phelypea* was inactive against the test bacteria,but it gave good antifungal activity against the yeast: *Candida albicans*.In the DPPH assay, the ethanol extract of *Cistanche phelypea* stems exhibited significant antioxidant activity close to that of the positive control propyl gallate

**KEYWORDS:** *Cistanch phelypea*, Isolation, Dihydroflavonol, Antibacterial Activity, Antioxidants Activity.

## INTRODUCTION

Herbal medicines have been massively used for remedy of diseases in traditional way for many generations.[1] Recently medicinal plants are extensively researched for new phytochemicals that could serve as leads for drug discovery and drug development taking in consideration the current concern of multi-drug resistance**.**

The flavonoids are secondary metabolites with interesting biological activity.[2,3] These plant phenolics exhibit a range of biological activities including: vasodilatory,[4,5] antitumor, anti-inflammatory, antibacterial, immune-stimulating, antiviral and antiallergic effects, as well as entity depressive of phospholipase $A_2$, cycloxyganase and lipoxyganase.[6-8]

*Cistanche* is a genus of about twenty species in the family Orobanchaceae.[9] The genus is widely distributed in Arica, Mediterranean region and Asia where it grows in arid and semi-arid areas.[10] Some of these species are widely used in ethnomedicne against various ailments including kidney disorders, infecions, infertility, constipation, profuse metrorrhagia[11] and inflammation.[12] They are also used as smooth muscle relaxant[13].Some phytochemicals like iridoids, lignans,phenylethanoid glycosides and alkaloids have been reported from Cistanche genus.[14-18]

*Cistanche phelypea* L. is a perennial plant. The plant is used traditionally against diabetes, diarrhea, infections, intestinal disorders and as diuretic.[19-21]

## MATERIALS AND METHODS

### Materials

### Plant material

*Cistanche phelypea* stems were collected from Omdorman, Sudan. The plant was authenticated by the Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum.

### Instruments

IR spectra were run on a Shimadzu IR spectrophotometer, UV spectra were run on a Shimadzu 2401PC UV- Visible Spectrophotometer. NMR spectra were measured on a Joel ECA 500MHZ NMR Spectrophotometer.

### Test organisms

The following standard microorganisms were used to assess the antimicrobial potential of *Cistanche phelypea* extract: *Bacillus subtilis* (Gram (+ve), *Staphylococcus aureus* (Gram(+ve), *Pseudomonas aeroginosa* (Gram –ve), *Escherichia coli* (Gram –ve) and the fungal species *Candida albicans*.

## Methods

### Preparation of plant extract for phytochemical screening

(200 g) Of powdered air- dried stems of *Cistanche phelypea* were extracted with 95% aqueous ethanol by maceration. This prepared extract (PE) was used for phytochemical screening. Phytochemical screening was accomplished according to the method described by Harborne.[19]

### Extraction and isolation of flavonoids

(1 kg) of powdered air-dried stems of *Cistanche phelypea* was macerated with 95% ethanol (5L) for 48hr at room temperature. The extraction process was repeated two more times with the same solvent. Combined filtrates were concentrated under reduced pressure at 40° C yielding a crude product.This crude product was applied on silica gel plates as narrow zones. The plates were developed with chloroform: methanol(9:1;v:v). After the usual workup a chromatographically pure flavonoid-compound I- was isolated.

### Biological activity

### Antimicrobial assay

By using the agar diffusion bioassay, *Cistanche phelypea* extract was assessed for antimicrobial activity Against four standard pathogenic bacteria and one pathogenic fungus: (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans*).

### Preparation of bacterial suspensions

Broth cultures of the test organisms were distributed into agar slopes and incubated at 37°C for 24 hours. Bacterial growth was harvested and suspended in 100 ml of normal saline to give about $10^8$-$10^9$ colony forming units(CFU) per ml. The Average number of viable organism per ml was determined using the surface viable counting technique.

Serial dilutions of the stock suspension were prepared in sterile normal saline. (0.02 ml) of the appropriate dilution was transferred into the surface of dried nutrient agar plates. After drying, the plates were incubated at 37° C for 24 hours. Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25°C for four days.

### Testing for antimicrobial activity

(2 ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45° C in a water bath. (20 ml) aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and each plate was divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No. 4).Agar discs were removed and cups were filled with (0.1 ml) of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37° C for one hour. Tests were performed in duplicates. After incubation the diameters of the resultant growth inhibition zones were measures and averaged.

For antifungal activity, instead of nutrient agar, Sabouraud dextrose agar was used. Samples were used here by the same concentrations used above.

### Antioxidant activity

### The DPPH radical scavenging assay

The ethanolic extract of *Cistanche phelypea* was dissolved in DMSO while DPPH was prepared in ethanol[22]. After incubation, decrease in absorbance was measured at 517nm. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group. All tests were run in triplicate.

## RESULTS AND DISCUSSION

### Characterization of compound I

Phytochemical screening of the stems of *Cistanche phelypea* unmasked the presence of tannins, flavonoids, saponin, steroids, terpenes and alkaloids.

From the ethanol extract, compound I was isolated by thin layer chromatography and its structure was elucidated via a combination of spectral techniques (UV, IR, $^1$HNMR).

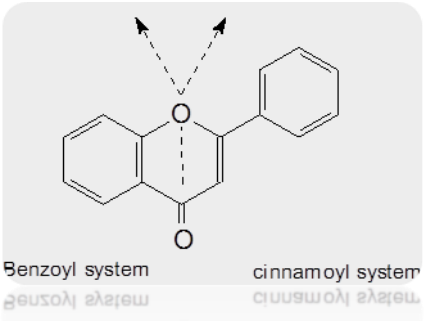
The IR spectrum of compound I (Fig.1) revealed ν (KBr): 675, 823 (C-H, Ar.,bending),1099 (C-O), 1454,1514 (C=C, Ar.),1704 (C=O), 2808 (C-H,aliph, stretching), 3303 $cm^{-1}$ (OH).

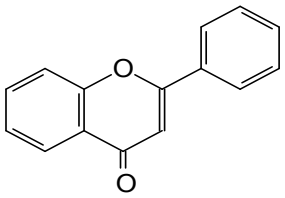

**Fig. 1: The IR spectrum of compound I.**

The UV spectra of flavonoid is an extremely useful data that can, in most cases, distinguish between the different classes of flavonoids. Flavonoids are classified into: flavones, flavonols, chalcones, aurones, flavanones, dihydroflavonols, dihydrochalcones, isoflavones, anthocyanins and flavans. Anthocyanins and flavans, unlike the other classes, are devoid of a 4-keto function and could easily be identified by the absence of the carbonyl stretching in their IR spectra as in the case of the IR spectrum of compound I, which failed to exhibit such stretching.

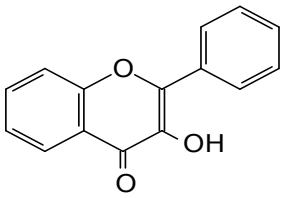
In their UV spectra, flavones, flavonols, chalcones and aurones show two absorption bands (the cinnamoyl band-called band I and the benzoyl band – called band II).The benzoyl absorption appears due to the extension of the carbonyl chromophore by ring (A) in the flavonoid nucleus, while the cinnamoyl absorption appears due to conjugation between the 4-keto function and the aromatic (B) ring of flavonoids as shown below. Band II occur in the range : 235-290nm, while band I appears in the range :300-400nm.

Isoflavones, flavanones, dihydroflavonols and dihydrochalcones give similar UV spectra as a result of little or no conjugation between the A- and B-rings. They typically exhibit an intense Band II absorption with only a shoulder or low intensity peak representing Band I. They are all readily distinguished from flavones, chalcones, aurones and flavonols which give both bands (I and II) due to conjugation between the $C_4$ carbonyl and the aromatic (B) ring.

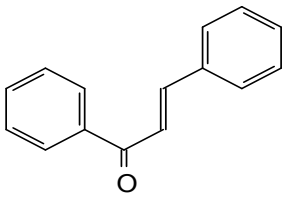


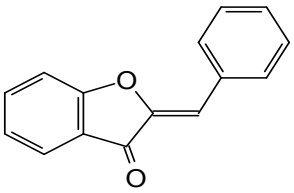
**Flavone**



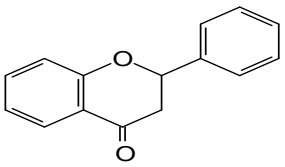
**Flavonol**



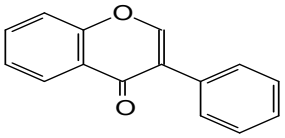
**Chalcone**



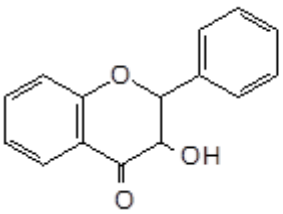
**Aurone**



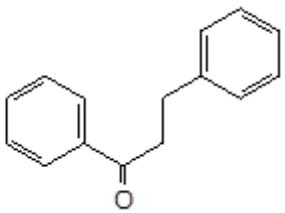
**Flavanone**



**Isoflavone**



**Dihydroflavonol**



**Dihydrochalcone**

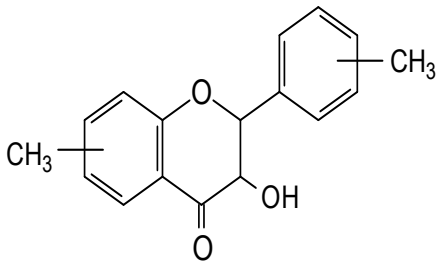
In the UV, compound I absorbs at ʎmax274 (Fig.2). Such absorption is characteristic of: flavanones, isoflavones, dihydrochalcones and dihydroflavonols. However, the characteristic shoulder of isoflavones- which appears in the range 300-340nm- was not detected in the UV spectrum of compound I. On the other hand, the sodium methoxide spectrum(Fig.3) showed a bathochromic shift with decrease in intensity suggesting the presence of a 3-OH which is a characteristic feature of dihydroflavonols. Hence compound I is a dihydroflavonol.

Different UV shift reagents were used to illustrate the hydroxylation pattern on the nucleus of the isolated dihydroflavonol; these are (i) sodium acetate(detects 7-OH group) and (ii) boric acid (detects catechols).

The sodium acetate spectrum did not reveal any bathochromic shift suggesting absence of a 7-OH group(Fig.4). Also the boric acid spectrum indicated absence of catechol moieties, it failed to show a bathochromic shift.

The $^{1}$HNMR spectrum of compound I (Fig.6) showed δ(ppm): 1.23, 1.61 assigned for two methyl groups. The aromatic protons resonated at δ6.65,6.80,7.38 and 7.95ppm. The signals at δ2.50 and δ3.40 ppm are due the solvent (DMSO) residual protons and residual water respectively.

On the basis of the above argument the following partial structure was proposed for compound I:




**Compound I**

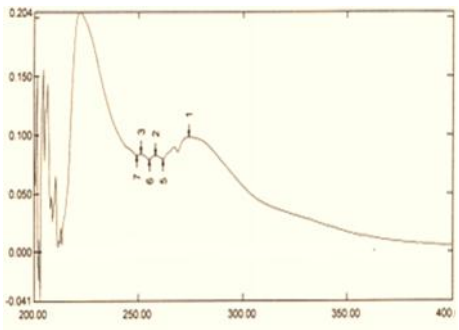


**Fig. 2: UV spectrum of compound I.**

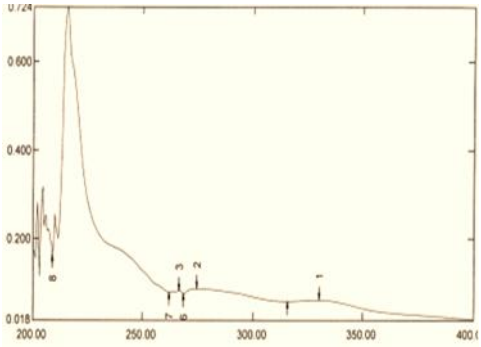


**Fig. 3: Sodium methoxide spectrum of compound I.**

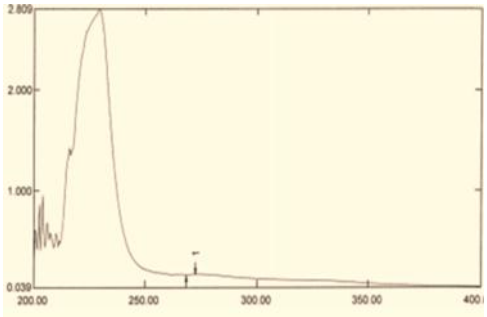


**Fig. 4: Sodium acetate spectrum of compound I.**

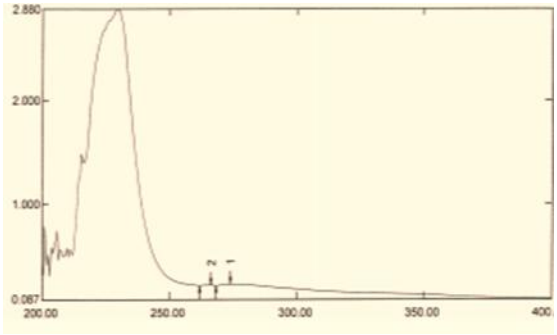


**Fig. 5: Boric acid spectrum of compound I.**

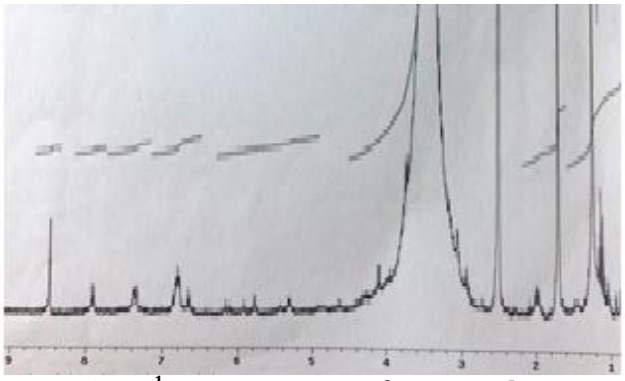


**Fig. 6: $^{1}$HNMR spectrum of compound I.**

**Antimicrobial activity**

The ethanolic extract of *Cistanche phelypea* was evaluated for antimicrobial activity against five standard microorganisms (Table 1). The results are depicted in Table (2). Results were interpreted in the following terms: (〉9mm: inative; 9-12mm: partially active; 13-18mm: active; 〈18mm:very active). Ampicilin,

gentamicin and clotrimazole were used as positive controls(see Tables 3 and 4).

The ethanolic extract of *Cistanche phelypea* was inactive against the test bacteria,but it gave good antifungal acivity against the yeast : *Candida albicans* (Table 2).

**Table 1: Test organisms.**

| No | Micro organism | Type | Sourse |
|---|---|---|---|
| 1 | *Bacillus subtillus* | G+ve | ATCC 2836 |
| 2 | *Staphylococcus aureus* | G+ve | ATCC 29213 |
| **3** | *Pseudomonas aeroginosa* | G-ve | NCTC 27853 |
| 4 | *Escherichia coli* | G-ve | ATCC 25922 |
| 5 | *Candida albicans* | fungi | ATCC 7596 |

* NCTC. National collection of type culture, Colindale. England
*ATCC. American type culture collection, Maryland, USA

**Table 2: Inhibition zones of ethanolic extract.**

| Samle | Conc.(mg/ml) | Sa | Bs | Ec | Ps | Ca |
|---|---|---|---|---|---|---|
| Ethanolic extract | 100 | --- | --- | --- | --- | 15 |

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

**Table 3: Antibacterial activity of standard drugs.**

| Drug | Conc.(mg/ml) | Bs | Sa | Ec | Ps |
|---|---|---|---|---|---|
| Ampicilin | 40 | 15 | 30 | - | - |
| | 20 | 14 | 25 | - | - |
| | 10 | 11 | 15 | - | - |
| Gentamicin | 40 | 25 | 19 | 22 | 21 |
| | 20 | 22 | 18 | 18 | 15 |
| | 10 | 17 | 14 | 15 | 12 |

**Table 4: Antifungal activity of standard chemotherapeutic agent.**

| Drug | Conc.(mg/ml) | An | Ca |
|---|---|---|---|
| Clotrimazole | 30 | 22 | 38 |
| | 15 | 17 | 31 |
| | 7.5 | 16 | 29 |

**Antioxidant activity**

The antioxidant capacity of the ethanol extract of *Cistanch phelypea* stem has been measured. Evaluation of the antioxidant activity was carried out by measuring the capacity of the test extract against stable DPPH radical. The change in color was measured spectrophotometrically at 517 nm. As displayed in (Table 5) the ethanol extract exhibited significant antioxidant activity close to that of the positive standard propyl gallate.

**Table 5: Radical scavenging activity of ethanol extract.**

| Sample | Antioxidant activity |
|---|---|
| Propyl gallate | 92.00% |
| Ethanol extract | 91.03% |

**REFERENCES**


1. Han. SS, Keum.YS, Seo.HJ, Surh.YJ, Curcumin suppresses activation of NF-_B and AP-1 induced by phorbol ester in cultured human promyelocytic leukemia cells, *J. Biochem. Mol. Biol.*, 2002; 35: 337.
2. El-Shemy. HA, Aboul-Enein. AM, Aboul-Enein.KM, Fujita. K., Willow stems' extracts contain anti-tumor agents effective against three cell types. *PLOS ONE*, 2007; 2(1): 178.
3. Harborne.J.B; Mabry, T, J.; Mabry, H, ""The Flavonoides", 1975, Chapman and Hall, London, UK.
4. Herrmann, K., flavonoides and flavones in plants: A. review. *J. Food Technol*., 1976, 11: 433-448.
5. Kuhnau, J.,The Flavonoides : A class of semi – essential food components : Their role in human nutrition, *World Rev. Nutr. Diet*, 1976; 24: 117-191.
6. Kandaswami. C; Middleton. E, Free radical scavienging and antioxidant activity of plant Flavonoides. *Adv. Exp. Med. Biol.*, 1994; 366: 351 - 376.
7. Ho. C-T., Chen. Q., Shi. H., Zhang. k.-Q. Rosen. R.T, Antioxidant effect of polyphenol extract prepared from various Chinese teas. *Prev. Med*., 1992; 21: 520-525.
8. Kinsella. J, E.Frankel, E.German, B. Kanner, J., Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol*. April, 1993; 85-88.
9. Duarte, J. Perez-Vizcainom, F. Utrilla, P. Jimenez, J. Tanargo, J. Zarzuelo, A., Vasodilatory effects of flavonoides in rataortic smooth muscle. Structure – activity relationships. *Gen. Pharmacol*., 1993; 24: 857-862.
10. Duarte, J.; Peres-Vizcaino, F.; Zarzueio, A.; Jimenez, J.; Tanargo, J., Vasodilator effects of quercetin in isolated rat vascular smooth muscle. *Eur. J. Pharmacol*., 1993; 239: 1-7.
11. Brown, J. P., A review of the genetic effects of naturally occurring flavonoides, anthroquinones and related compoundes. *Mutat. Res*., 1980; 75: 243-277.
12. Lindahl, M. Tagesson, C., Selective inhibition of groups 11 phospholipase $A_2$ by quercetin. *Inflammation*, 1993; 17: 573-582.
13. Tackholm, V., Students' Flora of Egypt, 2nd edn., 1974, Cairo Univ. Press, Beirut.
14. Endo. K, Takahashi. K, Hikino. H, Structure for forsythoside B, an antibacterial principal of Forsythia koreana stems, *Heteroycles*, 1982; 19: 261–265.

15. El-Shabrawy.OA, Melek.FR, Ibrahim. M, Radwan. AS,Pharmacological evaluation of the glycosidated phenylpropanoids containing fraction from Orobanche crenata., Arch Pharmacol Res., 1989; 12: 22–25.
16. Xiong.Q, Kadota. S, Tani.T, Namba.T., Antioxidative effects of phenylethanoids from Cistanche deserticola. Biol Pharm Bull, 1996; 19: 1580–1585.
17. Naran.R, Ebringerova. A, Hromadkova. Z, Patoprsty. V, Carbohydrate polymers from underground parts of Cistanche deserticola. *Phytochemistry*, 1995; 40: 709–715.
18. Sukhdev. S. H; Suman. P. S. K; Gennaro. L and Dev. D. R., Extraction technologies for medicinal and aromatic plants", 2008, United Nation Industrial Development Organization and the International Center for Science and High Technology, 116.
19. Harborne, J. B. "Phytochemical Methods". 2nd edition, 1984, Chapman and Hall, London, UK.
20. Martinez A, Valencia G., Marcha Fitoquimica: In Manual de Prácticas de Farmacognosia Fitoquímica, 1999. 1st edition, Medellin: Universidad de Antioquia.
21. Wall, M. E; Eddy, C. R; McClenna, M. L; and Klump, M. E., Detection and estimation of steroid and saponins in plant tissue. Analytical Chemistry, 1952; 24: 1337-1342.
22. Shimada. K, Fujikawa.K, Yahara. K, Nakamura.T, Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. J Agric food Chem., 1992; 40: 945-8.