Pharmacognostical, Phytochemical and Pharmacological studies on aerial parts of Cistanche tubulosa

Mohammad Kamil,F.Ahmad, El.T.Abdallah*
Zayed Complex for Herbal Research & Traditional Medicine
Department of Health, Abu Dhabi,UAE

Abstract:

Cistanche tubulosa (*C.tubulosa*) possess a wide spectrum of medicinal properties, especially for use in anti-senescence, antioxidant, neuroprotection, anti-inflammation, hepatoprotection, antineoplastic, anti-osteoporosis, anti-aging, promotion of bone formation and reduces impotence. This original article has comprehensive information on the aerial part of cistanche tubulosa covering the botanical, physico-phytochemical, macroscopic, microscopic, spectroscopic and pharmacological aspects in detail. These data will lay the ground for its correct identification and distinguishing it from other Cistanche species specially the Cistanche deserticola. These will also be useful to promote its clinical application as an aphrodisiac and anti-aging medicine. The standardization parameters and distinguishing characters enlisted in this study will ensure the efficacy, safety and will be helpful for the preparation of monograph of this holoparastic herb.

Keywords: Aphrodisiac, anti-aging, *Cistanche tubulosa*, elemental analysis, Gas Chromatography-Mass Spectrometry (GC-MS), microscopic, spectroscopic, thin layer chromatographic fingerprinting.

* *Corresponding author e-mail:drkamil55@hotmail.com*

Introduction:

Cistanche tubulosa (Schenk) Wight is a holoparasitic desert species in the genus *Cistanche* belonging to the Orobanchaceae family. It grows in the Hotan region, in the sparsely populated Hsinchiang Uighur Autonomous region of northwestern China, southern Russia, southern Mongolia, Gansu, Qinghai. It is common in coastal areas of the Arabian Gulf coast and on inland saline sand plains and desert regions of India and Iran. *C.tubulosa* was included to the Chinese pharmacopeia, 2005, as an alternative for its similar chemical constituents and pharmacological activities compared to *cistanche deserticosa*. The plant lacks chlorophyll and obtains nutrients and water from the host plants whose roots it parasitizes. (Wikipedia, the free encyclopaedia).

Cistanche tubulosa (Schrenk) is officially recorded as the dried succulent stems of Cistanche in Chinese Pharmacopoeia Commission (2015). *Cistanche tubulosa* has the capacity to improve memory, immunity, sexual ability, and reduce impotence, chronic renal disease, morbid leucorrhea, profuse metrorrhagia, senile constipation (Zhang 2005); Chinese Pharmacopoeia Commission (2015), (Zhiming Li 2016); (Zwe-Ling Kong 2018) and Antidepressant (Yang Li 2018). It is used as hepatoprotective, vasorelaxant, antioxidant, anti-aging and anti-inflammatory (Lin 2002), (Morikawa 2010) and for treatment of diarrhea (Ghazanfar 1994). *Cistanche tubulosa* acteoside and echinacoside constituents are effective as anti-aging (Chu-Yan,et al.,2017 and Ningqun et al., 2017).

The phytochemical analysis has revealed several main chemical constituents of *Cistanche* Herba, including phenylethanoid glycosides (PhGs), iridoids and iridoid glycosides, lignans, alditols, oligosaccharides, and polysaccharides (Cai H,2007,Jiang,et al.,2009). Echinacoside is present in many species of *Echinacea* but especially high percentage in *Cistanche tubulosa* (Cai H 2007). Echinacoside (one of the PhGs) and PhGs from *Cistanche tubulosa* found to protect dopaminergic neurons against dopamine (DA) neurotoxicity. The aerial parts of the plant contains alkaloids, coumarins, saponins, sterols (and/or terpenes) and tannins (Ghazanfar 1994). Syringin, β-sitosterol, daucosterol, 8-epiloganic acid, echinacoside, cistanoside A in whole plant. (Rastogi 1993, 1998). Fructose, Mannitol, Glucose and Sucrose also detected by NIR spectroscopy (Xinhong et.al 2017).

Pharmacological activities, such as neuroprotective, anti-aging (Ningqun et al., 2017), immunomodulatory, anti-inflammatory, hepatoprotective, and anti-oxidative are due to the high concentration of PhGs, present in *cistanche tubulosa* (Jiang and Tu, 2009; Fu et al., 2017). Nowadays Cistanches Herba is widely accepted as an herbal tonic for general debility (Fu et al., 2017). C. tubulosa glycoside capsules (Memoregain®) are in use for the treatment of Alzheimer's disease (Guo et al., 2013).

Most species belonging to *Cistanche* genus used as medicinal plants for millennia in China, have a reputation as a superior tonic, and are known as "Ginseng of the Deserts."

Material and Methods:

Collection and identification of plant material:

The aerial part of *Cistanche tubulosa* (Figure I) was collected from Mafraq area, Emirates of Abu Dhabi and identified by a taxonomist of Zayed Complex for Herbal Research and Traditional Medicine(ZCHRTM), Abu Dhabi, UAE., and preserved with a tag #2739 at the herbarium of ZCHRTM, Abu Dhabi, UAE.

Phytochemical:

Successive extraction with organic solvents:

The under shade dried plant material was made powder using cutting mill. Accurately weighed 20 grams of the powder was packed in a cellulose extraction thimble and extracted with 250 ml petroleum ether (60-80°C) using soxhlet extraction apparatus. The extraction was continued until the petroleum ether in the thimble became clear. After the complete extraction, the extract was filtered and the solvent was distilled off using vacuum evaporator. The obtained residue was dried in a vacuum desiccator over anhydrous sodium sulphate. The percent yield of the petroleum extract was calculated.

The left over mark was air dried to remove the solvent and was simultaneously extracted with chloroform and absolute alcohol successively. The yield was calculated in each case.

Preparation of extracts:

The acetone and methanol extracts were prepared from accurately weighed powder using accelerated solvent extractor system (Dionex ASE 200 accelerated solvent extractor) at low temperature and high pressure and dried in freeze dry system (Labconco Freeze Dry System attached with stoppering tray dryer). All these extracts were stored in a vacuum desiccator for different studies.

Physicochemical analysis:

The physicochemical parameters namely water and absolute alcohol solubilities,loss in weight on drying, total ash, water soluble and acid insoluble ash, and pH values were determined (WHO 2011; Evans 2002).

Pharmacognostical studies:

Macro and Microscopic characteristics:

The arrangements size, shape, base, texture, margin, apex, venation, color, odor and taste were observed. Microscopic study carried out on the thin transverse section using microtome razor. The photograph of the prepared slides taken using Leica microscope fitted with digital camera attached to a PC (Tyler V 1977).

Thin layer chromatography (TLC Fingerprints):

Silica gel 60 F254 coated Aluminum sheets from Merck, Germany were used. HPLC grade solvents were used in preparation of mobile phases to develop thin layer chromatograms (TLC) (Wagner H 1996).

Accurately weighed 0.050 gram of dried extract dissolved in 1 ml of methanol and 10 microliter of this solution were applied spot wise on the TLC plate using micro syringe.

The developed chromatograms were scanned under ultra violet lights using CAMAG Video Scan TLC/HPTLC evaluation system, Switzerland.

GC-MS Analysis:

GC-MS-QP2010 Ultra in combination with an auto injector (AOC-20i) system (Shimadzu Kyoto Japan) used for GC-MS analysis. The system was equipped with a mass selective detector with an ion source having temperature 280°C and interface temperature 280°C. Capillary Column used for MS analysis was Rtx 5ms capillary column with 30m x 0.25mm (length and diameter) x 0.25µm film thickness. The temperature of the injector was adjusted to 280°C, possessing a split injection mode. The initial temperature applied was 60°C (2- minutes), increased to 180°C (2minutes at hold) and then 300°C at ramp rate of 9°C and 13°C/minutes respectively. Helium with purity of 99.99% used as carrier gas with 47.2cm/second of linear velocity. The total flow programmed was 13.3ml/minutes with columns flow of 1.69-ml/minutes and systems pressure 100.00Kpa. The chromatogram was acquired in scan mode with scan speed1666. The mass range of 40-500m/z with 1000ev of threshold selected. The spectrum was interpreted based on databases of National Institute standards and technology (NIST 11 Lib) and Willey 8 Lib.

Elemental analysis of ash from air-dried material:

Accurately weight air-dried plant material was completely ashed at about 600°C. Ash was dissolved in a known volume of 0.50M Nitric Acid. Ash solution analyzed quantitatively for different elements present in the plant material using atomic absorption spectrophotometer attached to auto sampler (AA-6800 Shimadzu Kyoto Japan) in combination Hydride Vapor Generator (HVG-1) used for the analyses of elements using flame method.

Results:

Pharmacognosy & Phytochemistry:

Plant Material of Interest: Dried Stem

Whole Plant

Aerial Parts

Herbarium Specimen

Figure I : *Cistanche tubulosa* (Schenk) Hook.f.

Synonyms : *Cistanche lutea* auct. Non-Hoffmg. & Link: Wight ; *Cistanche tubulosa* var. tomentosa Hook. f.

Family : Orobanchaceae

Macroscopic characteristics:

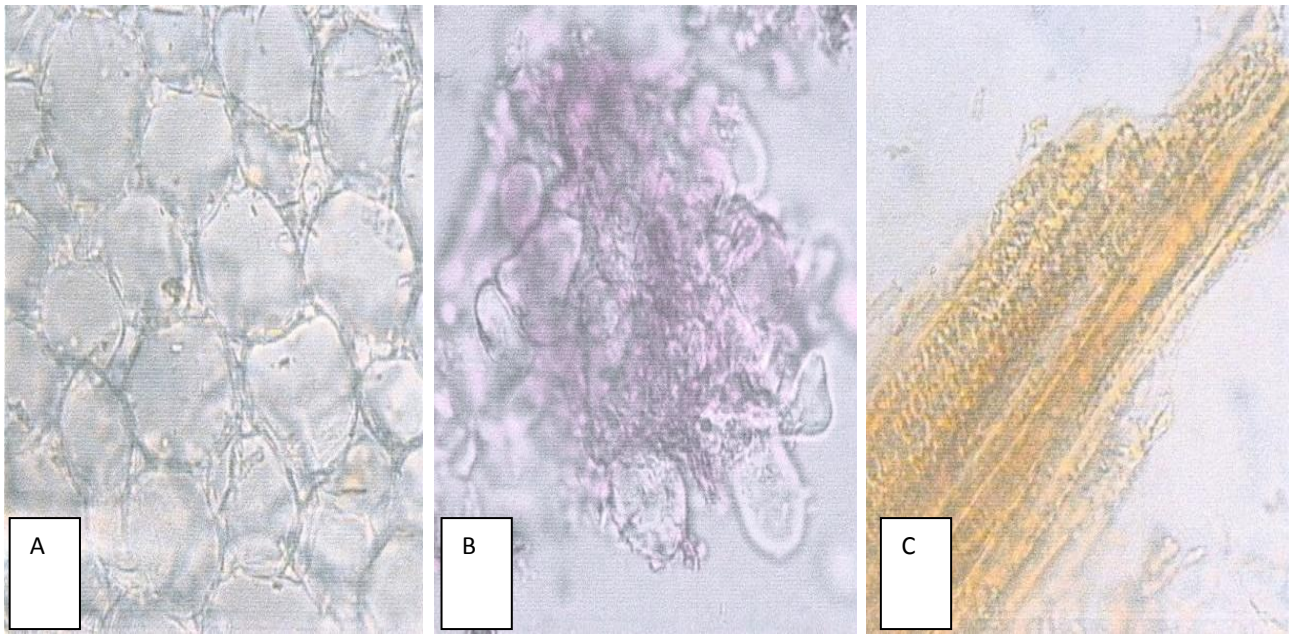
The stem is hard and woody in structure. It has almost a rectangular cross-section with significant ridges and a small 'hole' at the center. Dark violet to dark-brown, fleshy herb, 30-60 cm tall, often with a purplish tinge, simple, erect, glabrous to puberulous, often broader (up to 5 cm) at the base. Scales 2-3 cm long, 10-15 mm broad, triangular to broadly linear, acute. The bract oblong-lanceolate, often purplish, acuminate, slightly longer than the calyx; bracteoles shorter than the calyx, narrow, linear to sub lanceolate. Calyx 14-18 mm long, including lobes c. 1/3 as long as the tube; lobes laterally overlapping, with rounded apex and membranous margins. Corolla 3-5 cm long, 1.5-2 cm broad at the mouth, much narrower and tubular below, usually yellowish with purplish lobes, rarely whitish; lobes short, sub rounded, entire, reflexed. Staminal filaments woolly at base; anthers densely hairy, usually rounded or blunt at the ends. Capsules 20-25 mm long, ovoid-oblong, laterally compressed, beaked, many-seeded; seeds 1 mm long, pitted, dark-colored (Jongbloed 2003, eFloras, Mandeville, 1990). The stem powder is lightly brown with a rubber-like odour and a very bitter taste.

Microscopic Characteristics:

Microscopically, the powder shows light brown-colored phloem sieve tubes with oblique slits, dark grey thin compactly packed xylem vessels mostly annularly thickened and groups of shredded brown fibbers. There are also occasional light brownish-orange colored materials (Fig.IIA).

A transverse section of the stem (Fig. II-B) shows from the periphery inwards the following characteristics: The outline of the stem is Microscopic Characteristics: Microscopically, the powder shows light brown-colored phloem sieve tubes with oblique slits, dark grey thin compactly packed xylem vessels mostly annularly thickened and groups of shredded brown fibers. There are also occasional light brownish-orange colored materials. A transverse section of the stem shows from the periphery inwards the following characteristics: The outline of the stem is irregular and the epidermal layer that consists of anticlinal compactly arranged oblong cells shows some undulations. The cortex consists of many layers of distorted polygonal collenchymatous cells, which are brown in color, and they have thick walls. The vascular bundles (Fig.II-C) consist of groups of phloem and xylem tissues separated by narrow medullary rays; xylem vessels are almost annularly thickened with few spirally thickened vessels and they are all heavily lignified while some of the tracheids are slightly lignified. The pith consists of rounded cells with thick walls and sometimes the cells separate from each other, forming spaces.

Parts studied Stem



Figures: IIA, IIB, IIC:

II- A- surface view of stem pith.

II- B-TS of the stem. A fragment of tissues associated with whitish separable scaly masses.

II-C -A fragment of light brown-coloured adjacent vascular (tissues) vessels mostly annularly and spirally thickened.

Physicochemical Constants (percentage):

The following physicochemical parameters were carried out (Quality Control Methods, 2011; Evans, 2002) on the stems of the plant *Cistanche tubulosa*, (ZCHRTM unpublished work):

Loss of weight on drying at 105°C : 11.50

Methanol extract : 27.45

Absolute alcohol : 10.80

Water extract : 40.00

Successive Extractives (percentage)

Petroleum ether (60-80°C) : 0.70

Chloroform : 1.60

Absolute alcohol : 22.30

Ash Values (percentage)

Total ash : 14.60

Water-soluble ash : 9.30

Acid insoluble ash (10% HCl) : 1.00

pH Values (aqueous solution)

pH of 1% solution : 6.569

pH of 10% solution : 5.845

Table-I Elemental Analyses of the ash (prepared from air dried powder of plant material)

Ash values (BH- Pharmacopeia -Reference)					
Assay and identification of metal (AOAC International- Reference)					
Apparatus	(AA-6800 Shimadzu-Flame method)				
Element	Std. conc. µg/ml(ppm)	Sample conc.mg/ml	Samples absorbance	Actual conc. mg/ml	Actual conc. (%)
Cr	1, 2, 4	20	0.0087	0.008515	0.0008515
Zn	0.25, 0.5, 1	20	0.6587	0.032125	0.003212
Cu	1, 2, 4	20	0.0360	0.010175	0.0010175
Fe	1, 2, 4	20	0.5364	0.225785	0.0225783
Pb	1, 2, 4	20	0.0018	0.002705	0.000275
Cd	0.25, 0.5, 1	20	0.0065	0.0003	0.00003
K	1, 2, 4	1	1.5115	13.601	1.3601
Ca	5, 10, 20	1	0.0301	5. 6467	0.56467

1ppm conc. = 1µg/ml, Actual conc. (%) =Actual conc. (ppm) x0.0001 [1ppm=0.0001%]

Table –II Ultraviolet (UV) Spectral Studies:

Ultraviolet Spectrum (USP reference)				
Apparatus	Milton Roy Spectronic Genesys 5 Spectrophotometer - Milton Roy.			
Sample conc. (mg/ml)	Solvent	λmax (nm)	λ min (nm)	Abs.(λmax - λ min)
1.015	Intestinal Fluid simulated without pancreatic pH=7.5±0.1	325 289	265 -ve	0.969 - 0.775 0.880
0.99	Gastric Fluid simulated without pepsin pH =1.2±0.1	325	262	0.928 - 0.601

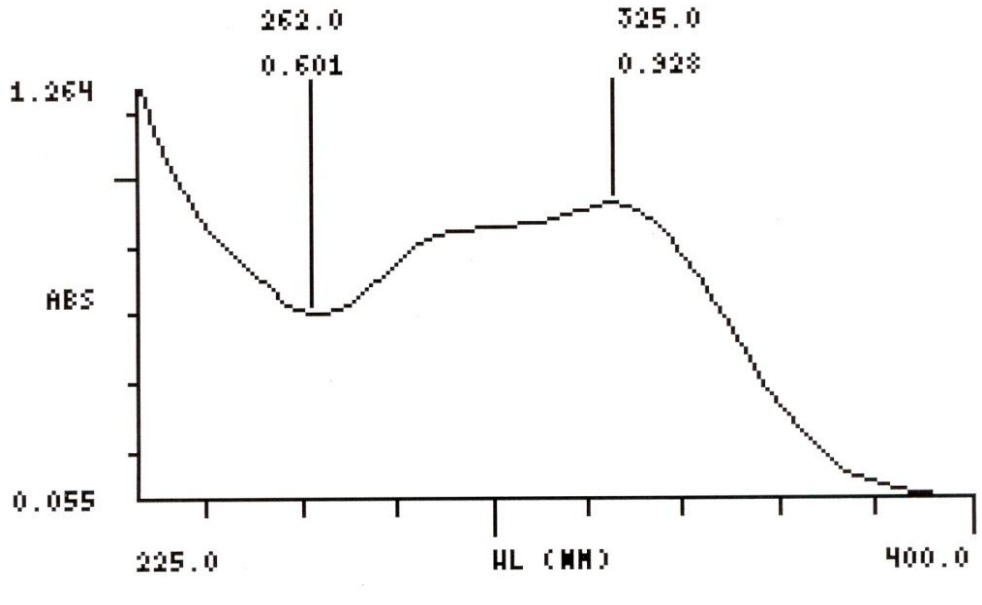


Figure III Intestinal Fluid simulated without pancreatic pH=7.5±0.1

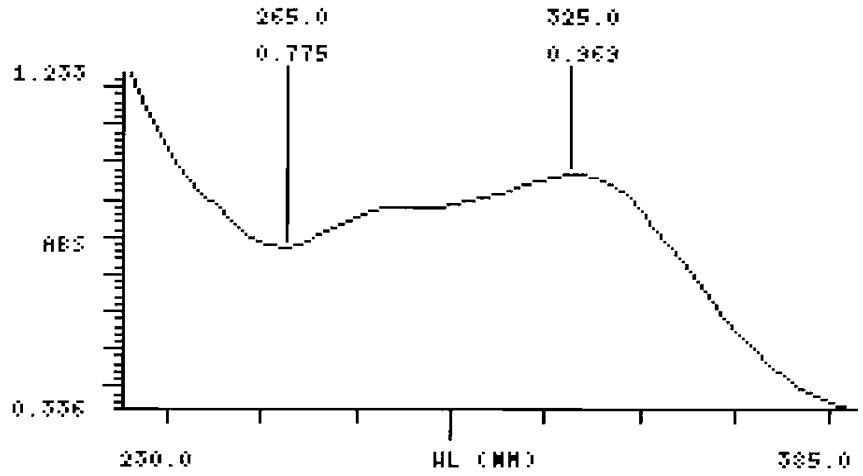


Figure –IV Gastric Fluid simulated without pepsin pH=1.2±0.1

Thin Layer Chromatographic Fingerprint (TLC):

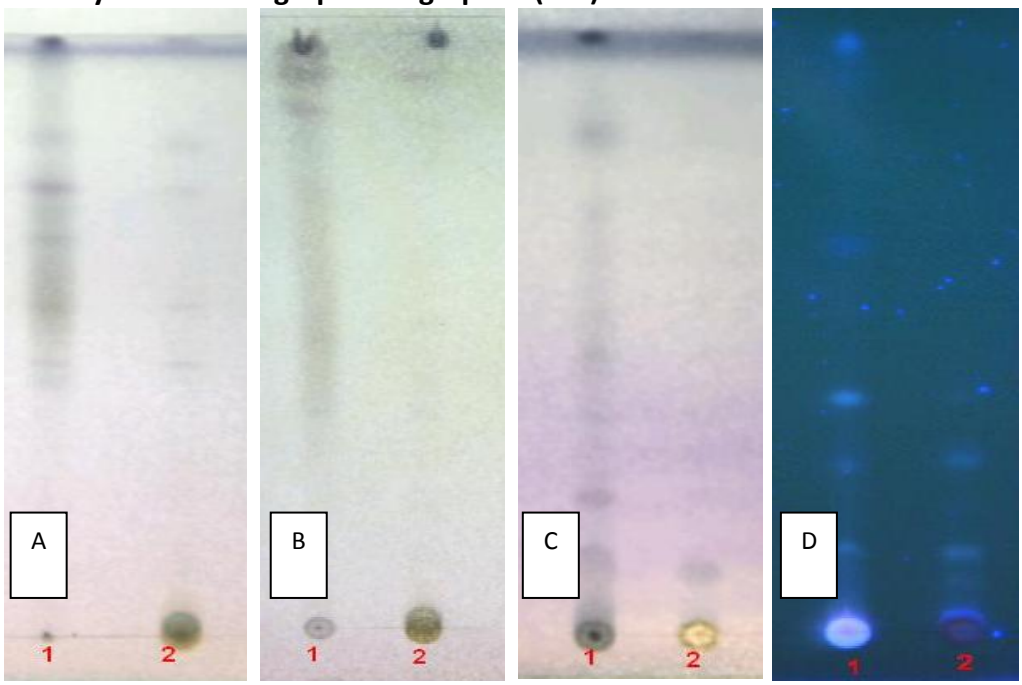


Figure- V TLC fingerprint of Pet. ether (60-80°C) extract (track 1) and MeOH extract (track 2)

Mobile phase	Fig. A: Toluene, ethyl formate, formic acid (5:4:1)
	B: Ethyl acetate, methanol, water (100:13.5:10)
	C & D: Toluene, ethyl acetate (93:7)
Derivatization	Fig A, B, & C: Vanillin-Sulphuric acid -Vis
Detection	Fig. D : UV 365nm

Reference standards were not used in TLC fingerprint.

GC-MS Analyses Result

Petroleum ether (60-80)(Fig-VI), acetone (Fig-VII) and methanol(Fig.VIII) extracts of aerial parts of *C.tubulosa* were qualitatively analyzed by GC-MS. Compounds namely, n-Pentacosane, Hexadecanol, Beta sitosterol, Oleyl alcohol, Tricasane, 9-Eicosene€, n-nonadecene, Behenic alcohol, Di-n-Octyl phthalate, Glycerin, Oleic acid, Tetratriacotane, Tetracontae, Heneicosane, Bis-(dimethylamino)methane, 4-Vinyl-2-methoxyphenol, d-Mannitol-1,4-anhydro, Mome inositol, Methylpamitate were identified.

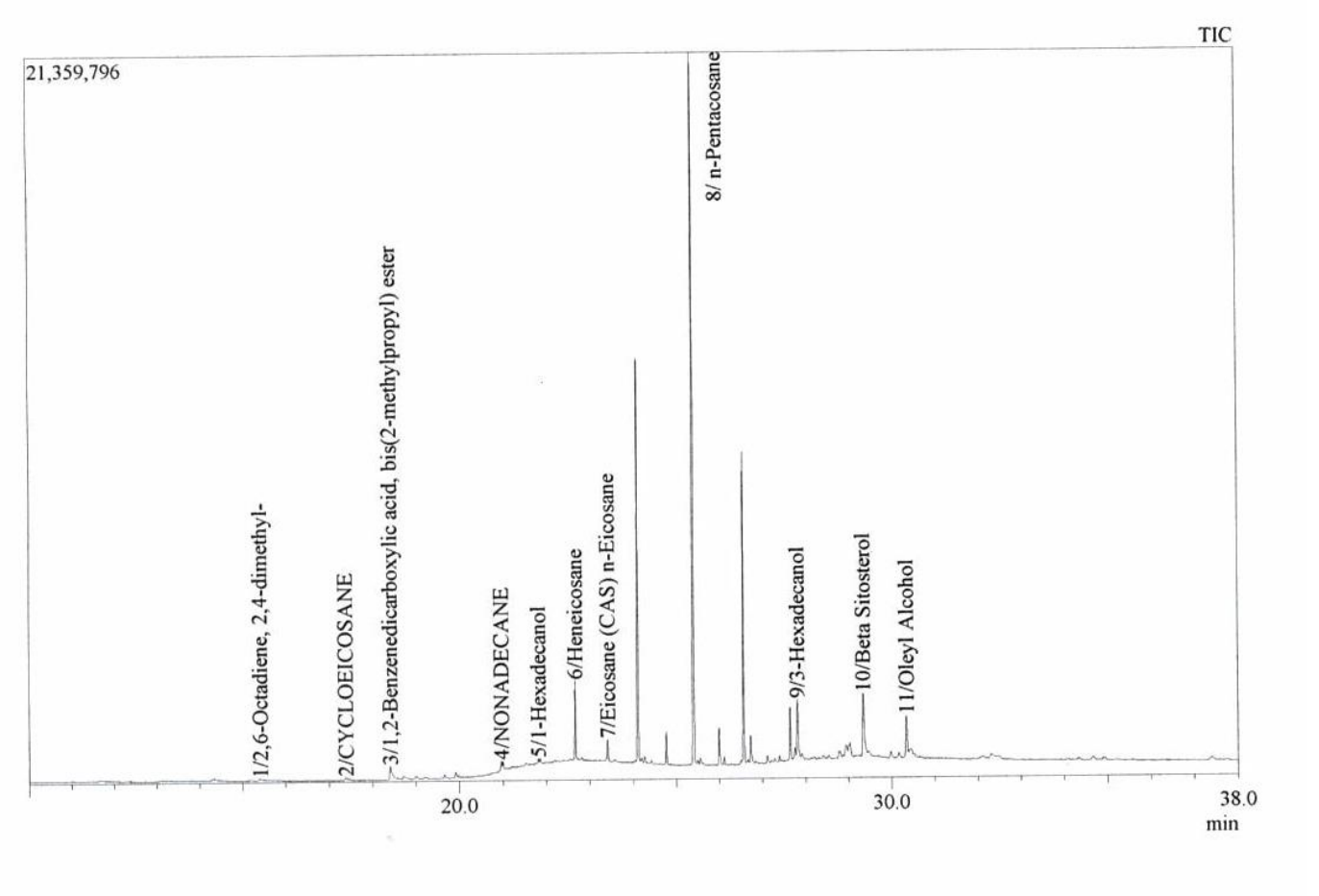


Figure –VI GC Chromatograph of Petroleum ether (60-80°C) extract of *Cistanche tubulosa*

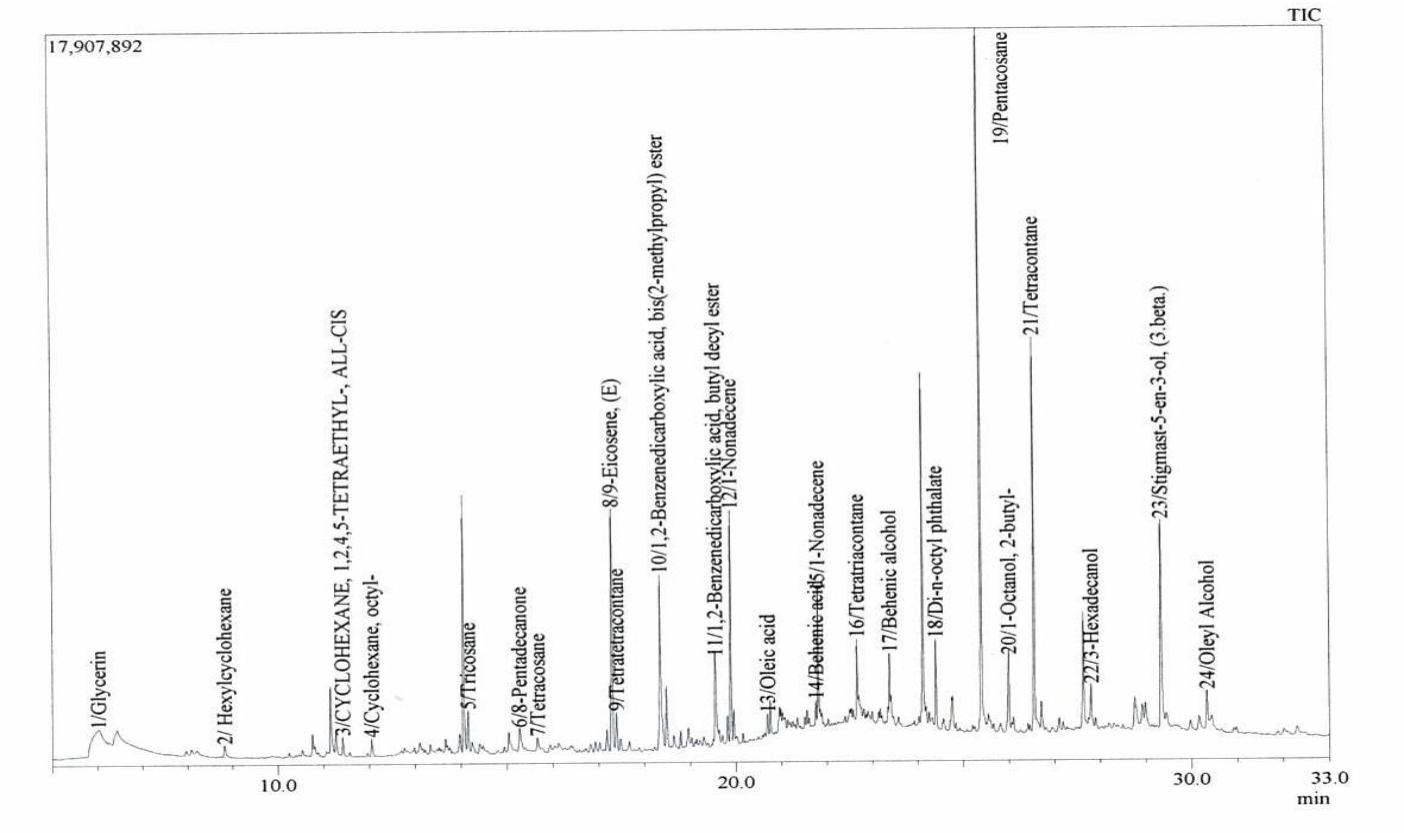


Figure-VII GC Chromatograph of Acetone extract of *Cistanche tubulosa*

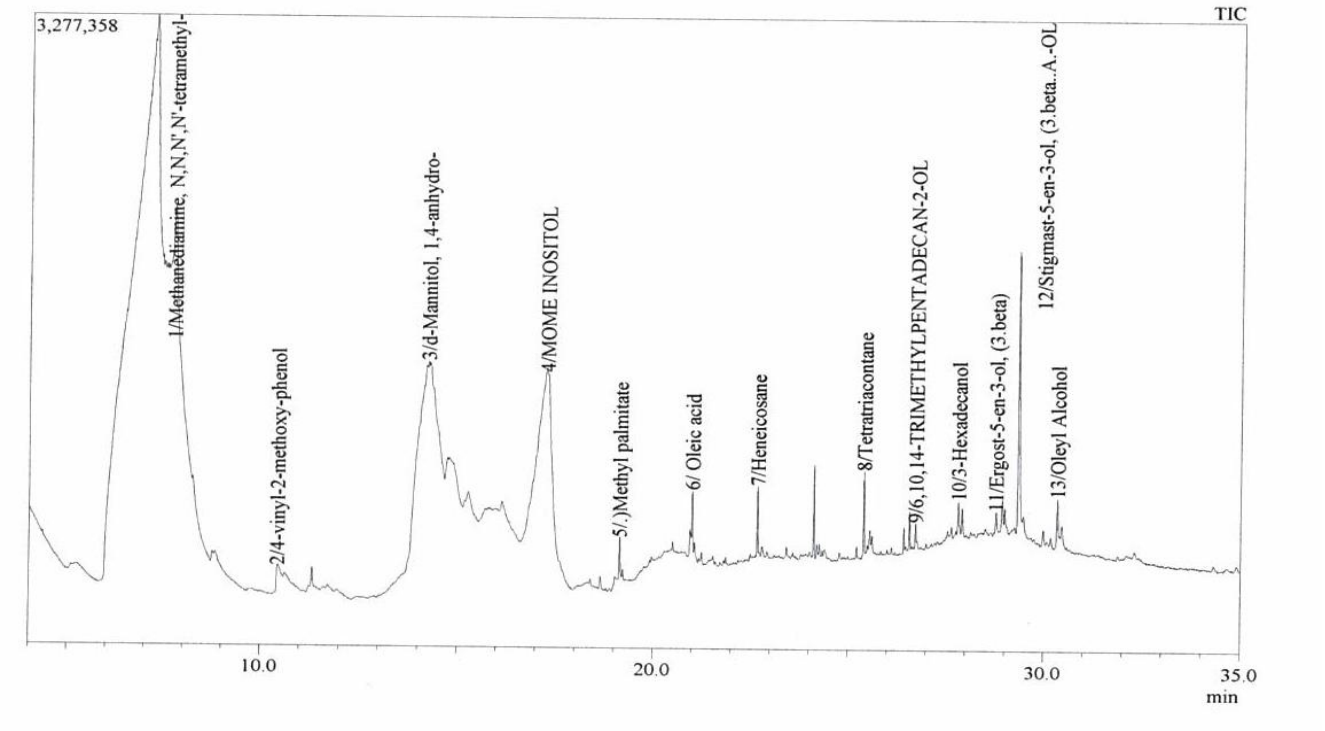


Figure-VIII GC Chromatograph of Methanol extract of *Cistanche tubulosa*

Pharmacological and Toxicological Studies:

Aqueous extract:

Shoots and stems of *Cistanche* species used as a food and tonic in traditional Chinese medicine for the inefficiency of the kidney. The whole plant of *Cistanche tubulosa* .used medically in Pakistan as a remedy for diarrhea and sores (Kobayashi*,* 1987)

A novel monoterpene from *Cistanche salsa*, a Chinese herb, reported to be an anti-osteoporotic compound (Yamaguchi, 1999). The phagocytic function of intra-abdominal macrophage in mice was activated by the decoction of C. deserticola and *Cistanche tubulosa* and increased the weight of the seminal vesicle and prostate gland (Zong, 1996).

Four phenylethanoids isolated from the stems of *Cistanche deserticola,* acteoside (1), 2'-acetylacteoside, isoacteoside, and tubuloside B, significantly suppressed NADPH/CCl4-induced lipid peroxidation in rat liver microsomes (Xiong, 1998).

Methanol extract:

The methanol extract from the dried stems of *Cistanche tubulosa* (Schrenk) R. Wight was found to show an inhibitory effect on contractions induced by noradrenaline in isolated rat aortic strips showing vasorelaxant activity (Yoshikawa 2006).

The attenuation of the serious behavioral disorder and increasing DA (dopamine) levels in the striata of MPTP-lesioned (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in mice was observed showing the neuroprotective effects of phenylethanoid glycosides from the *Cistanche salsa* using Parkinson's disease model in both rodents and primates (Geng 2004).

Acteoside from *Cistanche salsa* improved cell viability and prevented the MPP (1-methyl-4-phenylpyridinium ion) induced apoptosis and inhibited the apoptosis-related pathway (Pu, 2003). It is reported that the phenylethanoid glycosides of the plant can prevent the 1-methyl-4-phenylpyridinium ion-induced apoptosis in cerebella granule neurons and exert its anti-apoptosis effect by inhibiting caspase-3 and caspase-8 activities (Tian 2005).

The following pharmacological & safety evaluation studies were carried out on the aqueous extract of the plant *Cistanche tubulosa* (Derelanko 2002*;* Han 2003).

Table III- Pharmacological & safety evaluation studies

ACTIVITY	**RESULTS**			
	Strong	**Moderate**	**Mild**	**Negative**
Analgesic				√
Anti-stress	√			
Anticonvulsant				√
Diarrheal	√			
Effect on rabbit jejunum	√			
Effect on Guinea pig ileum	√			
Effect on rat fundus		√		
Effect on right rat atria	√			
BP & HR, Carotid artery method	√			
Effect on Guinea pig tracheal chain			√	
Hepato-protective				√
Utrotropic				
Biochemical profile				√
Motor co-ordination (grip strength & motor activity				√
Rectal temperatu				√
Body weight				√
Vital organs				√

Summary of the Results for Table-III.

The aqueous plant extract administered at a single dose showed significant analgesic activity, both central and peripheral. The extract administered orally showed diarrheal effect. The results revealed the spasmogenic activity of the plant, and designate the purgative nature of the plant extract in relieving constipation.

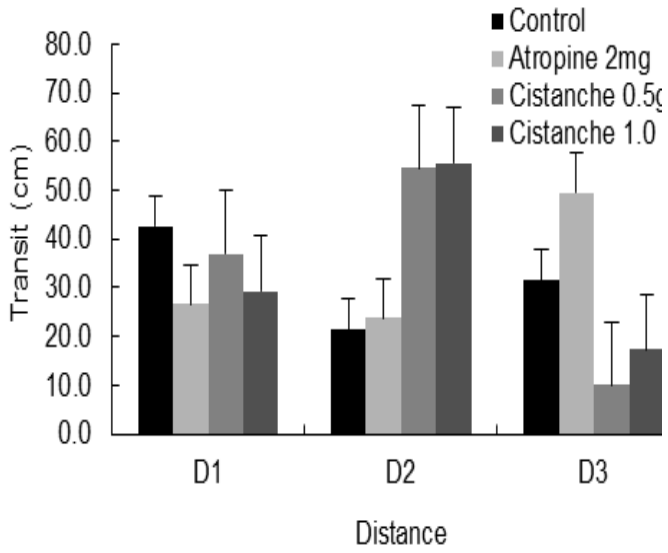


Figure-IX The effects of *Cistanche* *tubulosa* aqueous extract 0.5, 1.0 g. /kg. and atropine 2mg/kg p.o. on gastrointestinal motility test in rats. D1= the distance from pylorus to beginning of plug. D2= the distance from the end of plug to caecum. D3= Total Distance from pylorus to caecum (D1 +D2).

The plant aqueous extract showed augmentation of smooth muscles, which may contribute to the laxative effect. However, the overdose may produce gastrointestinal disturbance, upset stomach or diarrhea. The plant extract was also found to increase the percentage of gastric emptying and motility. Thus, from this study, it appears that the plant extract can serve as a useful alternative to prokinetic drugs. The plant extract showed significant anti-inflammatory properties, and was showed to act as a cardio protective /myocardial stimulant, along with having a tonic effect on the heart. The plant extract showed significant reduction of systolic or diastolic blood pressure and heart rate; it also showed anti-stress activity/ adaptogenic activity and mild anticonvulsant activity. No significant hepatoprotective activity was observed.

The single oral dose of the test of the plant did not produce any adverse effect in the general behavior and gross appearance in experimental mice. The drug did not show any sign of salivation, nasal discharge, diarrhea or constipation, loss of righting reflex, lacrimation and tremors. The LD_{50} was assumed> 6.4 g/kg. The aqueous extract of *Cistanche* appears to be safe in mice at a dose of 6.4 mg/kg when given orally.

Discussion:

Correct identification of a plant and its' standardization is crucial to maintain efficacy. Parameters for Standardization of a crude drug and its extracts include physicochemical constants, Macroscopic and Microscopic studies, phytochemical, TLC fingerprinting, and spectroscopic studies. Quantitative elemental analysis of crude drug is crucial for its toxicity. Evaluating all these parameters after identification of the plant, will ensure and help in maintaining quality, purity, efficacy and toxicity of the plant as drug.

It is important to evaluate the physicochemical constants of plan crude drug as they help in identifying adulterants and or improper handling of the plant material.The result of loss on drying was 11.50% indicating that the plant drying process was efficient. This is an important parameter since it determines the efficiency of drying process, which in turn indicative of the stability of the drug storing time. If drying is efficient, it will not encourage the growth of decay causing microorganism. The total ash, water-soluble ash and acid insoluble ash values are indicative of the purity of crude plant. The high value of total ash indicates the presence of elements and or dusty materials in high concentrations. If total ash is high and acid insoluble ash is low, it indicates that high value of total ash is due to high concentration of inorganic elements present in crude drug High percentage of acid insoluble ash indicates the presence of dusty maters, which in turn indicates that crude plant was not cleaned properly. The results of total ash and acid insoluble ash values are 11.05% and 1.00% respectively. These data indicate that the *Cistanche tubulosa* contains minerals in good concentration. Extractive values indicate the nature, type and amount of chemical constituents present in the crude drug. The extractive value was maximum in water and minimum in petroleum ether. High solubility of chemical constituents of the crude drug in water indicates the presence of high concentration of glycosides, carbohydrates and inorganic minerals. Ultraviolet spectra (UV), TLC and GC-MS chromatograms of plant extract act as fingerprints for identification and as well as identification of chemical constituents present in the plants. UV spectra, TLC chromatograms and GC-MS analysis of methanol extract have been done. These data will act as fingerprints for *C. tubulosa.* Qualitative elemental analysis of crude plant for some elements has been carried out. These data along with toxicological study result will play a very important role to determine the safety of the crude plant as a drug. Powder, Macroscopic, and Microscopic Studies (Pharmacognostic studies) are essential for every plant, which provide the characteristic feature of that plant. They act as reference standard and are consider as diagnostic feature of that particular

plant. The results of powder, macro and microscopic studies described in details in the macro and microscopic characteristics part.

Conclusion

The Phytochemical, Pharmacognostical, and different Spectroscopic data enlisted in this study will lay the ground for its' correct identification and will differentiate from the other *Cistanche* species, especially from Cistanche deserticola as both closely resembles to each other. These data will also be useful to promote its clinical application as an aphrodisiac and anti-aging (Life span elongation) medicine. Standardization parameters and toxicology data laid down will ensure the efficacy and safety of the herb as a drug. The all data including the pharmacological and toxicological studies will also be helpful in preparation of monograph on this holoparasitic herb.

References:

Bauer A.W., Kirby W.M., Sherris J. C., & Turck M. (1966) *Antibiotic susceptibility testing by a standardized single disk method*. Am J Clin Pathol, 45:493–6.

British Herbal Pharmacopoeia (1996). 4th Ed, British Herbal Medicine Association (BHMA).

Cai H, Bao Z, Jiang Y, Wang XY, Fan ET, Aierken M.,(2007). Study on processing method of Cistanche tubulosa, Zhongguo Zhong Yao Zazhi, 32:1289-1291

Derelanko M. J., & Hollinger M. A., (2002). *Handbook of Toxicology*. (2nd ed.). Boca Raton, USA, CRC Press.

El-Ghonemy A.A., (1993). *Encyclopedia of Medicinal plants of the United Arab Emirates*. (1st Ed.). Abu Dhabi, UAE, United Arab Emirates University.

Evans W.C., (2002). Trease and Evans *Pharmacognosy,* (15th ed., Elsevier A division of Reed Elsevier Indi PVT. Limited).

Flora of Pakistan. e-floras, Web.

Fu Z, Fan X, Wang X, Gao X. (2017). Cistanche herba; an overview of its' chemistry, pharmacology, and pharmacokinetics properties. J.Ethanopharmacol. 219:233-247

Geng X., Song L., Pu X., & Tu P., (2004). *Neuroprotective effects of phenylethanoid glycosides from Cistanches salsa against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced dopaminergic toxicity in C57 mice*. Biol Pharm Bull, 27:797-801.

Ghazanfar S. A., (1994). *Handbook of Arabian Medicinal Plants*. Boca Raton, USA: CRC Press.

Guo Q., Zhou Y., Wang C.J., Huang Y. M, Lee Y.T., Su M.H., et al.(2013). An open-label nonplacebo-controlled study on *Cistanche tubulosa glycosides capsules (Memorigain) for treating moderate Alzheimer's disease. Am .J. Alzheimer's Dis .Dement 28: 363-370*

Han J., & Hoosier, G. L. V. J., (2003). *Handbook of Laboratory Science, Animal Models*. (Second ed., Vol. II). USA, CRC Press.

Jiang y and Tue P-F. (2009).Analysis of chemical constituents in Cistanche species. T. Chromatogr. A, 12161:1970-1979.

Jangled M.V.D., Feulner G.R., Boer B., & Western A. R., (2003). *The Comprehensive Guide to the Wildflowers of the United Arab Emirates*. Abu Dhabi, UAE: ERWDA.

Kobayashi H., Oguchi H., Takizawa N., Miyaze T., Veno A., Usmanghani K., & Ahmed M., (1987). *New Phenylethanoid glucosides from Cistanche tubulosa* (SCHRENK) HOOK. f. Part I. Chem Pharm Bull 35:3309–3314

Lin L. W., Hsieh M. T., Tsai F. H., Wang W. H., & Wu C. R., (2002). *Anti-nociceptive and anti-inflammatory activity caused by Cistanche deserticola in rodents.* Journal of Ethnopharmacology, 83:177-82.

Mandeville J. (1990). *Flora of Eastern Saudi Arabia*. London, UK. Kegan Paul International Ltd.

Masayuki Yoshikawa, Hisashi Matsuda, Toshio Morikawa, Haihui Xie, Seikou Nakamuraa, & Osamu Muraoka., (2006). *Phenylethanoid oligoglycosides and acylated oligosugars with vasorelaxant activity from Cistanche tubulosa*. Bioorganic & Medicinal Chemistry 14 7468–7475.

Morikawa T, Pan Y, Ninomiya K, Imura K, Matsuda H, Yoshikawa M, et al.,2010. Acetylated phenylethanoid oligoglycosies with hepatoprotective activity from desert plant Cistanche tubulosa. Bioorg Med Chem.18:1882-1890.

Mothana R. A. A., Abdo S. A. A., Hasson S., Althawab F. M. N., Alaghbari S. A. Z., Lindquist U., (2008). *Antimicrobial, Antioxidant and Cytotoxic activity and Phytochemical Screening of Some Yemeni Medicinal Plants*. Evidence- Based Complementary and Alternative Med. 7(3):323-30.

Official Methods of Analysis of AOAC International (1999). 16th Ed. Vol. I and II

Pharmacopoeia of the People's Republic of China, (2005). The chemical industry Publishing House, Bejing.

Pu X., Song Z., Li. Y., Tu P., & Li H., (2003). *Acteoside from Cistanche salsa inhibits apoptosis by 1-methyl-4-phenylpyridiniumion in cerebellar granule neurons*. Planta Med., 69:65-6.

World Health Organization (2011) Quality Control Methods for Medicinal Plants Materials , Geneva.

Rastogi & Malhotra, (1993). *Compendium of Indian Medicinal Plants*. (Vol. 4, p187). New Delhi, PID.

Rastogi & Malhotra, (1998). *Compendium of Indian Medicinal Plants*,Vol. 5:, p210). New Delhi: PID.

Shi H.M., Wang J., Wang M.Y., Tu P.F., Li X.B., (2009). *Identification of Cistanche Species by Chemical and Inter-simple Sequence Repeat Fingerprinting,* Biological and Pharmaceutical Bulletin, 32(1), 142-146

Tian X.F., Pu X.P., (2005). *Phenylethanoid glycosides from Cistanches salsa inhibit apoptosis induced by 1-methyl-4-phenylpyridinium ion in neurons.* Journal of Ethnopharmacology 97:59-63.

Tyler V, Brady L, Robber T. (1977). J. Pharmacognosy, Varghese Company, India, 103-141

Wagner, H., & Bladt, S. (1996). *Plant Drug Analysis-A Thin Layer Chromatography Atlas*. (2nd Ed.) Springer-Verlag, Berlin Heidelberg.

Wang X., Li L., Muhuyati, & Du N., (1998). *Anti-oxidative action of the glycosides of Cistanche in the tissue of mice*. Zhongguo Zhong Yao Za Zhi, 23:554-555.

WHO, 1998.Xiong Q., Hase K., Tezuka Y., Tani T., Namba T., & Kadota S., (1998). *Hepatoprotective activity of phenylethanoids from Cistanche deserticola.* Planta Med, 64:120-125.

Yamaguchi K., Shinohara C., Kojima S., Sodeoka M. , & Tsuji T., (1999). *(2E, 6R)-8-hydroxy-2, 6-dimethyl-2-octenoic acid, a novel anti-osteoporotic monoterpene, isolated from Cistanche salsa.* Biosci Biotechnol Biochem, 63:731-735.

Yang Li, Ying Peng, Ping Ma, Hanlin Yang, Haiyan Xiong, Mengyue Wang, Chongsheng Peng, Pengfei Tu, and Xiaobo Li(2018). Antidepressant like Effects of Cistanche tubulosa Extract on Chronic Unpredictable Stress Rats through Restoration of Gut Microbiota Homeostasis. Frontiers in Pharmacology, 9:967.

Yoshikawa M., Matsuda H., Morikawa T. , Xie ,H., Nakamuraa S., & Muraoka O., (2006). *Phenylethanoid oligoglycosides and acylated oligosugars with vasorelaxant activity from Cistanche tubulosa*. Bioorganic & Medicinal Chemistry, 14: 7468–7475.

Yoshikawa M., Matsuda H., Morikawa T., Xie H., Nakamura S., & Muraoka O. (2006). *Phenylethanoid oligoglycosides and acylated oligosugars with vasorelaxant activity from Cistanche tubulosa*, Bioorganic & Medicinal Chemistry 14:746.

ZCHRTM unpublished work.

Zhang CZ,Wang Sx,Zhang Y,Chen JP and Liang Xm,(2005).In Vitro estrogenic activities of Chinese medicinal plants traditional used for managements of menopausal symptoms. Ethanopharmacol.98:295-300.

Zhiming Li, Huinuan Lin, Long Gu, Jingwen Gao, and Chi-Meng Tzeng (2016). Herba Cistanche (Rou Cong-Rong): One of the Best Pharmaceutical Gifts of Traditional Chinese Medicine. Frontiers in Pharmacology, 7:41

Zong G., He W., Wu G., Chen M., Shen X., and Shi M., (1996). *Comparison between Cistanche deserticola Y. C. Ma and C, tubulosa (Shenk) Wight on some pharmacological actions.* Zhongguo Zhong Yao Za Zhi., 21: 436-7.

Zwe-Ling Kong, Athria Johnson, Fan-Chi Ko ,Jia-Ling He, and Shre-Chun Cheng,(2018). Effect of cistanche tubulosa extract on male reproductive function in streptozotocin nicotinamide indused diabetic rats. Nutrients, 10(10):11562.