Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Pharmacognistic, proximate and phytochemical analysis of stem of *Cistanche tubulosa* (Schenck) Hook. F.

Maryum Nousheen[1], Abdul Basit[2*], Muhammad Kamran Khan[3], Rasha M. Alzayed[4], Sondos A. Alhajouj[4], Meaad F. Alaida[4], Awatif M.E Omran[5], Abdulrahman Alasmari[5], Ahmad El Askary[6], Riyadh S. Almalki[7], Mohamed M. Zayed[8,9*]

[1] *Deportment of Botany Islamia College Peshawar, Pakistan*
[2] *Department of Botany Islamia College Peshawar, Pakistan*
[3] *Department, of Biotechnology and Genetic Engineering, Hazara University, Mansehra, Pakistan*
[4] *Biology Department, College of Science, Jouf University, Sakaka 41412, Saudi Arabia*
[5] *Department of Biochemistry, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia:*
[6] *Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P.O.Box 11099, Taif 21944, Saudi Arabia;*
[7] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Umm AL-Qura University, Makkah, Saudi Arabia*
[8] *Department of Chemistry, Rabigh College of Sciences and Arts, King Abdulaziz University, Jeddah 21589, Saudi Arabia*
[9] *Environmental and Occupational Medicine Department, National Research Centre, Cairo 12622, Egypt*

Article Info

Article history:

Received: October 17, 2023
Accepted: December 19, 2024
Published: December 31, 2024

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Abstract

A medicinal plant is any plant that in one or more of its organs contains substances that can be used by it or their constituent for therapeutic purposes. The present work was done to evaluate pharmacognostic, fluorescence, proximate and phytochemical analysis of ethanolic extracts of *Cistanche tubulosa* (Orobanchaceae) along with antimicrobial activity. Antimicrobial activity against four bacterial strains *S. aureus*, *E. coli, P. aeruginosa, S. typhi* along with five fungal strains such as *F. oxyfurum, P. notatum, Candida albicans, A. fumigatus,* and *A. niger* evaluate using agar well diffusion method. The powder drug study of various tissues of plants revealed higher concentrations (20.29 mg/l) of essential macro and micronutrients. Fluorescence analysis of the stem powdered with various chemical reagents showed different colors. Proximate analysis showed the presence of crude substances such as proteins (8.5 %), fat (1.5 %), fibres (6.6 %), carbohydrates (73.87 %), moisture contents (3.23 %) and ash contents (6.3 %) respectively. Phytochemical screening revealed the presence of carbohydrates, proteins and dozen other important secondary metabolites. The presence of these bioactive constituents associated with the antimicrobial activity of *S. aureus* showed the maximum zone of inhibition (15.1 ± 3.7 mm), while in antifungal activity *C. albicans* showed the highest zone of inhibition (11.0 ± 3.15 mm). The pharmacognostic study, fluorescence analysis and antimicrobial activity are helpful in the standardization of the drug establishing a good support for the use of *C. tubulosa* in traditional medicine.

Keywords: Orobanchaceae, Pharmacognostic constituents, Fluorescence analysis, Proximate analysis

1. Introduction

A medicinal plant is any plant that in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. The plants contain a wide variety of phytochemicals such as tannins, terpenoids, alkaloids, and flavonoids that dictate the therapeutic potency of the plants most especially the antimicrobial activities. They are considered a rich source of substances that can be used for the synthesis of drugs [2]. Traditional medicine has been used by the majority of the world population for thousands of years. Studies have been carried out globally to verify their efficiency and some of the findings have led to the production of plant-based medicines [3]. Recent focus on plant research has increased worldwide and most evidence has been collected to determine the immense potential of medicinal plants [4]. Approximately 414,000 flowering plants have been reported from the world, out of which 40,000 have been used for medicinal purposes [5]. While about half of the population in industrialized countries uses herbal medicines [6]. The parasitic *Cistanche* has not only remarkable value in oases development but also a bright prospect in economic development because *Cistanches herba* (CH) are well-known medicinal plant and nominated as a “ginseng of the deserts” [7]. The dried stems of Cistanche genus consist of *Cistanche salsa*, *Cistanche*

* Corresponding author.
E-mail address: abdulbasiticp@gmail.com (A. Basit): mmzaid@kau.edu.sa (M.M. Zayed).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.12.4

Deserticola, *Cistanceh Sinensis* and *Cistanche Tubulosa* are not only well-known edible plants but also well-known medicinal plants. According to the Japanese medicinal history the dried stem of *Cistanche salsa* and *Cistanche tubulosa* are enlisted as a Crude Drug"Nikujuyou"[8].

The majority of the world diverse medicinal plant cultivation countries export their products to the non-diversified ones stabilizing their economic situation and alternate needs. According to the Export Promoting Bureau, there was an export of over 8,500 tonnes of medicinal herbs in 1999, which fetched a petty amount of 6 million US dollars and Pakistan stands among the eight prominent countries, that export medicinal plants [4]. Over 700 plant species are utilized for medicinal purposes in Pakistan, with the majority being uni-regional, while only a few are bi-regional or pluri-regional [9]. The present work was conducted to evaluate pharmacognostic feature of *Cistanche tubulosa* of Orobanchaceae [10]. The selected plants were collected from district Karak of KPK bounded on the North by District Kohat and Hangu, and on the South by District Lakki Marwat Districts on the main Indus highway between Peshawar and Karachi. The district of Mianwali is located to the southeast, bordered by the district of Bannu and the Waziristan Agency to the west [11]. The district consists of 103 species belonging to 43 families. The dominant families were Fabaceae (11 spp.), Asteraceae and Poaceae (10 spp.) each, while the rest of the families show variable numbers of species each

Cistanche tubulosa, a parasitic plants that occur in district Karak KPK may also reported from northern areas of Pakistan and Kashmir. Six species were found in Balochistan, and only two in Sindh and some species are also present in, Sibi, Las Bella, Mianwali and in Sindh and in Nara Desert [12]. The *Cistanche tubulosa* grows as a parasite on roots of *Calotropis* and *Salvadoran* species that are abundantly found in West Asia, North African states and Arab Countries as well [13]. The World Health Organization (WHO, 1998) has described guiding principles for the standardization of medicinal plants regarding their macroscopic and microscopic description [14]. Standardization of herbal formulations is essential in order to assess quality of drugs, based on the concentration of their active principle, physical, chemical, physio-chemical standardization and in-vitro, in-vivo parameters. However, the macroscopic elaboration shows certain ambiguity due to manual contamination. Therefore, an extensive anatomical, physicochemical and phytochemical screening was required that is helpful to avoid any ambiguity [15].

2. Materials And Methods

2. 1. Collection and preservation

The plants of *Cistanche tubulosa* were collected in April 2019 from a deserted area in Karak, Khyber Pakhtunkhwa during the flowering season. After a thorough survey of the Karak Division plants was collected from three different localities of District Karak viz., (i) village Bogara via Amberri Ada (ii) Gangai on Indus Highway near Hamadan Chowk (iii) Thal Wazir on Bannu Link road. The underground stems were separated from the roots and washed carefully to remove debris. The collected plants were oven-dried at 65ºC for 72 hours and identified with the help of the flora of Pakistan. An electric grinder was used to make stem powder of 50g and stored in a tight bottle to use for powder drug study, extractive value, elemental analysis, nutritional composition, phytochemical screening, and antibacterial and antifungal activity.

2. 2. Extraction

Extraction was done by dissolving the powder of *Cistanche tubulosa* stem in different solvents namely ethanol, methanol, chloroform, n-hexane, and acetone but the best results were obtained in ethanol and it gave the extractive value of (14%). The ethanolic extract was achieved by soaking 30g of powdered underground stems in 300 ml of ethanol. The conical flask was air-tight with aluminium foil, properly labelled, and stirred at room temperature and for 168 hours (7 days). The extracted sample was filtered with Whatman filter paper into a separate 100 ml conical flask. The extract was concentrated by evaporating at 50 °C in a water bath so as to concentrate it to 15 ml. The concentrated extract was poured into 3 ml glass bottle and used for various pharmacognostic, elemental analysis, phytochemical analysis, nutritional composition and antimicrobial activities [16],[17].

2. 3. Powder drug study

2. 3. 1. Macroscopic evaluation

Organoleptic evaluation is an important step for the identification of any adulterant in the medicinal plant. It refers to sensory evaluation. Fresh part of the underground stem was taken and characteristics were evaluated such as color, odor, size, taste, shape and texture [18].

2. 3. 2. Microscopic evaluation

The powder of stem of *Cistanche tubulosa* was stained with respective reagents such as hydrochloric acid, iodine solution and chloral hydrate. The sample was mounted on glass slide and observed under the microscope (×10 and × 40) to determine the type of cells, phloem fibres and lignified tissues [19].

2. 3. 3. Elemental analysis

A small amount of 0.5g stem powder was dissolved in 10 ml of nitric acid and allowed to stand overnight followed by 4ml perchloric acid and it was boiled on a hot plate in a fume hood. After some time yellowish color of the plant material (stem) changed into white fumes that indicate digestion was completed. After cooling 100ml of distilled water was added to the extract. Followed by filtration and the samples were labeled carefully [20]. The filtered samples were analyzed through Atomic Absorption Spectroscopy (AAS) and atomic absorption spectrophotometer [21].

2. 3. 4. Determination of extractive value

The dried powdered underground stem of *Cistanche tubulosa* was extracted with n-hexane, methanol, ethanol, acetone and chloroform using a maceration process. One gm of the coarsely powdered plant material was weighed in a weighing bottle and transferred into a 250 ml conical flask. Then the flask was filled with different solvents (20 ml) separately. The flasks were corked and kept aside for 24 hours at room temperature, and shaken frequently. The mixtures were filtered through Whatman No. 1 filter paper into a 50 ml measuring cylinder. The faltered was then transferred into weighed Petri dishes and concentrated by complete evaporation [22].

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

2. 3. 5. Fluorescence analysis

A small quantity of the stem powder was on clean microscopic slide adding 1-2 drops of freshly prepared different reagent solutions mixed by gentle tilting and waiting for a few minutes. Now the slide was placed inside the UV chamber and observed in visible light, of 254nm-366nm radiations. The colors observed by the application of different reagents in different radiations were recorded [23].

2. 4. Nutritional Analysis (Proximate analysis)

The proximate analysis (fats, proteins, moisture, carbohydrates and ash) plant was determined by using AOAC methods [24],[25].

2. 4. 1. Determination of moisture contents

Petri plates were autoclaved for 35 minutes at 105C°. In a desiccator, the Petri plates were cooled and then weighed. The moisture content was measured and described by the AOAC method. One gram sample was placed in a preheated and weighed glass Petri plate and then dried in a hot air oven at 130 °C for 2 hrs or till constant. Weight after drying the glass Petri plate and was transferred to the desiccator to cool and then Petri plate was reweighed. The loss in weight was calculated as [26].

$$\text{Moisture content (\%)} = \frac{\text{Fresh sample weight (w1) \_ dry sample weight (w2)}}{\text{Weight of sample}} \times 100$$

2. 4. 2. Determination of ash contents

To determine the ash content, oven method was used. One gram of the sample was added to a preweighed crucible and weighed. Then, the sample was placed in a muffle furnace at 550 °C for 4 h, until it was white, indicating the absence of carbon cooled in desiccators and reweighed. The ash content was determined by using the following equation [27].

$$\text{Ash} = \frac{W1 - W2}{\text{Weight of sample}} \times 100$$

W1 = weight of sample+ crucible
W2 = weight of sample after being kept in the furnace

2. 5. Determination of crude fat

Soxhlet apparatus following the standard procedures of AOAC, 1984 were used [28]. A sample of 3g of powder enveloped by filter paper was placed in a thimble and transferred to the extraction chamber of the apparatus. A dry clean 250 ml round bottom flask was weighed (w1) and filled with 150 mL of petroleum ether connected with an extraction tube containing thimble. The thimble was placed in an extractor covering anti-bumping cotton, fixed the extractor to a pre-weighed oil flask [27]. The flasks were heated at 50 °C and the Soxhlet apparatus ran for 5 h. The thimble filters were carefully removed. A rotary evaporator was used to dry the extract by complete evaporation and then weighed (w2). The % fat was calculated as [29].

$$\text{\% fat} = \frac{W1 - W2}{\text{Weight of original sample}} \times 100$$

W1 = weight of flask with fat
W2 = weight of empty flask

2. 6. Determination of crude fibers

About 2 gm. of moisture and fat-free sample was weighed and transferred to the spoutless one-liter beaker. Thereafter, 200 ml 1.25% H_2SO_4 was added and the beaker was placed on a hot plate to reflux for 30 mins, timed from the onset of boiling shaking the content for every 5 min. The beaker was removed and the contents were filtered through a muslin cloth. The residue was washed with hot water till it was free from acid.

2) The alternate procedure was used in the same beaker with the same concentration of NaOH solution for 30 mins. The filtered residue was washed with hot water till it was free from alkali. The total residue was transferred to a crucible and placed in a hot air oven, allowed to dry to a constant weight at 80-110 °C and weighed. The residue was ignited in a muffle furnace at 550- 600 °C for 2-3 hrs, cooled and weighed again. The loss of weight due to ignition was the weight of crude fiber [30].

$$\text{\% of Crude Fiber} = \frac{W1 - W2}{\text{gm of the sample taken}} \times 100$$

W1 = Wt of the crucible with dry residue
W2 = Wt of crucible with ash

2. 7. Protein Determination

Crude protein was determined using the Kjeldahl apparatus. The determination of protein in plant samples is categorized into three stages:

2. 7. 1. Digestion of the powder

In a digestion flask, 1gm of the dried powder along with 2gm of the digestion mixture (potassium sulphate, ferrous, copper sulphate: 7:1 (w/w) were taken and then added to the flask having 12 ml of concentrated H_2SO_4 and mixed thoroughly by spinning. The solution was placed on a digester to boil, then cooled and digested and about 30 ml distill water was added in 5 ml portions with mixing and transferred into a 100 mL volumetric flask and made the volume up to the mark.

2. 7. 2. Distillation of the powder

The Kjeldahl apparatus was assembled, and 5 mL of the digest was added to the distillation tube. Next, 10 mL of 40% sodium hydroxide solution was introduced into the digest. After 10 minutes, the distillation process was completed, and the ammonia (NH_3) produced was collected in the receiving flask as ammonium hydroxide (NH_4OH).

2. 7. 3. Titration of the powder

To the flask 20 ml solution of 4% boric acid (H_3BO_3) and some drops of methyl red indicator were added. In the presence of trapped ammonia the addition of 0.05 N-hydrochloric acid to boric acid. When the color of boric acid

changes to pink the presence of ammonia will be confirmed. The pink color slowly transformed into yellowish color because of NH_4OH during distillation. For the calculation of protein amount, the following formula was used.

$$\%\text{of protein} = \frac{4.38 \times 1.4 \times 0.1\text{NHCL} \times \text{Vol of } H_2SO_4\text{(USED)}}{\text{Weight of sample}} \times 100$$

Where 4.38 = Protein factor for cereals,1.4 = Weight of Nitrogen in grams [31].

2. 8. Determination of carbohydrates

Carbohydrate was determined when the sum of the percentages of moisture, ash, crude protein, and crude fat was subtracted from 100 [32].

Total carbohydrates content = 100 % - [% moisture + % crude fats + % crude proteins + % ash +% fibers].

2. 9. Phytochemical analysis

The stem ethanolic extract was tested for the absence or presence of various constituents namely alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponins, glycosides, steroidal glycosides, proteins, fixed oil and fats, resins, antraquinones and terpenoids [20].

2. 10. Biological Activities (Antimicrobial Activity)

2. 10. 1. Antibacterial activity

The effectivity of natural extracts and pure compounds against various bacteria is noticed by observing the growth of bacteria in the presence of plant samples that are placed in close contact with them. The following activities were carried out to see the antibacterial potential of stem extracts of *Cistanche tubulosa* against four bacterial strains using the good diffusion method. Three strains *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* were gram-negative and *Staphylococcus aureus* was gram-positive. Antibiotic Ciprofloxacin was used as positive control. The zones of inhibition of crude drugs were measured after 24 hours of incubation at 37C and compared with the inhibition of standard drugs ciprofloxacin. All the data are expressed as mean ± standard deviation [33].

2. 11. Antifungal activity

2. 11. 1. Fungal strains

Five fungal strains were obtained from the Department of Chemistry, Agriculture University Peshawar which originated from clinical cases. These fungi were *Aspergillus niger, Fusarium oxyfurum, Penicillium spp, Candida albicans, Aspargilus fumigatus* were first grown on Potato Dextrose Agar (PDA and then inoculated for testing. Solution of a standard antibiotic (Flucanozole) was used as positive control. The antifungal bioassay was done by using the agar well diffusion method. After solidification of the media, wells of 6 mm were dug in media by using sterile plastic borer. Each well was given a specific number. Using a micropipette, Wells were loaded differently by stock solution (30 µg/mL), 50 µg /mL, 100 µg/mL and 150 µg/mL and labelled. Fungal plates were incubated at 37C°for 72 h. The diameter of zone of inhibition was measured. Each experiment was done in triplicate and mean values were taken [34],[35].

$$\%\text{ inhibition of fungal growth} = 100 - \frac{\text{linear growth and test (mm)}}{\text{linear growth in control (mm)s}} \text{ X } 100$$

2. 12. Statistical analysis

Data are provided as mean ± SEM. SPSS 20.0 software was used to conduct one-way ANOVA on various groups. A p-value of less than 0.05 indicated a significant difference.

3. Results

3. 1. Powder Drugs Study

Organoleptic evaluation is an important step for the identification of any adulterant in the medicinal plant. It refers to sensory evaluation. A fresh part of the underground stem was taken and characteristics were evaluated such as color, odor, size, taste, shape and texture [18].

3. 2. Extraction value

Extraction was done by dissolving the powder of *Cistanche tubulosa* stem in different solvents namely ethanol, methanol, chloroform, n-hexane and acetone. The best results were obtained in ethanol solvent and it gave the extractive value of (14%) which showed the highest concentration followed by methanol with 13%. The lowest extraction value was recorded with solution of chloroform representing a total of 1% of the extracted value (Fig1).

3. 3. Fluorescence analysis

Fluorescence analysis of powder drugs study revealed that each reagent shows variable fluorescence depending on the visible wavelength. Changing the wavelength of visible light alters the color observed. However, an important factor to consider is that the solution used in the procedure can also cause variations, as it absorbs different wavelengths, as shown in Table 2. The solution containing amide ions exhibits greater darkness at a wavelength

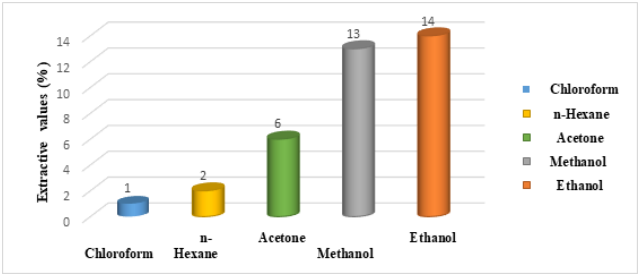

Fig. 1. Showing extractive values of *Cistanche tubulosa* (Schenk) Hook.f. stem.

Table 1. Powder drug study of *Cistanche tubulosa* (Schenk) Hook.f. stem.

Colour	Taste	Odour	Texture	Fracture
Light Brown	Acrid	Unique Characteristic Smell.	Smooth	Soft

of 366 nm compared to the lighter color of sulfide ions, which appear brown at the same wavelength. However, only slight differences were observed in both cases of the reagents under lower wavelengths. Additionally, other solvents displayed variable colors in the powdered drug study of Cistanche tubulosa, with different wavelengths shown in Table 2.

3. 4. Phytochemical analysis

Various tests were performed using the powder drugs of *Cistanche tubulosa* mandatory for drug evaluation. Representing the nature and identification of carbohydrates Molisch test was mostly used. Using the concentration of sulphuric acid with powder drugs of the stem of *C. tubulosa* a reddish color represents the presence of carbohydrates [36]. However, the nature of carbohydrates can be determined using the Fehling test, which is employed for the detection of reducing sugars. Equal amounts of Fehling A and Fehling B reagents were mixed with the extract and boiled until the color changed to brick red, indicating the presence of reducing sugars (Table 3) [37]. Similarly, Benedict's reagent was used to represent reducing sugar with brown-red precipitation (Table 3) [38]. Other tests for Alkaloids (Wagner's test, Dragendroff's [39], Bontrager's for Glycosides [40], Killere Killiani test for glycosides [39] and Froth test for Saponins [41] are represented in Table 3.

3. 5. Proximate analysis

The proximate analysis (fats, proteins, moisture, carbohydrates and ash) plant was determined by using AOAC methods [42],[25]. Proximate analysis of *Cistanche tubulosa* revealed Carbohydrates with the highest concentration say 74.47% [32] while the crude fats represent the lowest concentration of 1.5%. Other contents like crude protein, Ash, Crude fibers, and moisture contents represent 8.3%, 6.3%, 6.2%, and 3.23% concentration respectively (Fig 2).

3. 6. Elemental analysis

All living organisms require inorganic elements Mg, Mn, Zn, K, Ca and many others for their growth and survival. Medicinal plants contain considerable amounts of mineral constituents, prerequisite for the correct growth and development of plants [43]. Elemental analysis of stem of *Cistanche tubulosa* in (Fig. 3) revealed the presence of macro and microelements. Eleven essential metals were screened but one of the metals, (Pb) was not detected while Ten (Mg, Zn, Fe, Ca, Na, Mn, Co, K, Ni, Cd) were detected and calcium, potassium and magnesium and iron were found in appreciable levels. Elements like copper, zinc and sodium are present in lower quantities (Fig. 3). The concentrations of all the elements are within the permissible limits set by WHO 1996 [44],[45]. In *Cistanche tubulosa* stem iron was in the highest concentration ha-

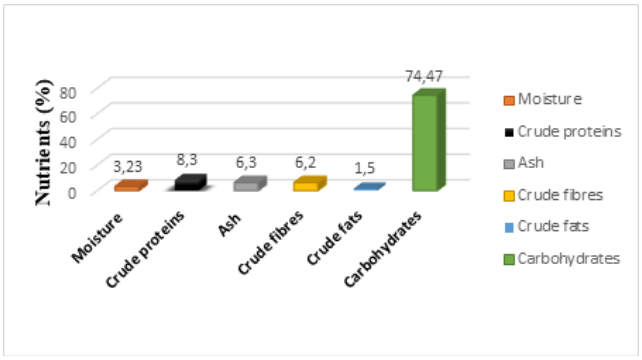

Fig. 2. Proximate analysis of *Cistanche tubulosa* (Schenk) Hook.f. stem.

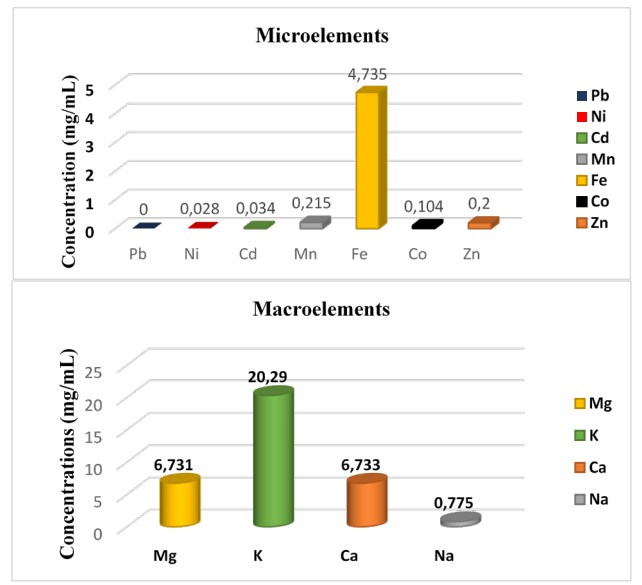

Fig. 3. a-b. Mineral composition of *Cistanche tubulosa* (Schenk) Hook.f. stem.

Table 2. Fluorescence analysis of *Cistanche tubulosa* (Schenk) Hook.f. stem.

S.No	Reagents	Visible light	UV 254 (nm)	UV 366 (nm)
1	Powder as such	Light Brown	Light brown	Dark Brown
2	Powder + con. HNO_3	Brown	Olive green	Dark brown
3	Powder +con. NH_3	Greenish black	Green	Dark brown
4	Powder +con.H_2SO_4	Black	Green	Brown
5	Powder + NaOH	Reddish Brown	Dark brown	Dark green
6	Powder +dil. HCL	Blackish brown	Light black	Dark green
7	Powder + Methanol	Light brown	Light brown	Dark brown
8	Powder + Ethanol	Dark brown	Blackish	Dark green
9	Powder + Iodine solution	Brown	Blackish green	Dark brown
10	Powder + $FeCl_3$	Yellowish brown	Dark brown	Black
11	Powder + Acetone	Brown	Light brown	Dark brown
12	Powder + Hexane	Dark brown	Greenish Black	Black

Table 3. Phytochemical analysis of *Cistanche tubulosa* (Schenk) Hook.f. stem.

Plant constituents	Tests performed	Ethanolic extract
Carbohydrates	Molisch's test	++
	Fehling's test	++
	Benedict's test	++
	Xanthoprotein test	+
Protein & amino acid	Biuret test	+
	Million's test	+
Alkaloids	Wagner's test	—
	Dragandroff's test	-
Glycosides	Borntrager's test	++
Steroidal glycosides	Killere killiani test	+++
Test for saponins	Froth test	+++
Test for flavonoids	Alkali test	+
Test for tannins	Ferric chloride test	+++
	Lead Sub acetate test	+++
Test for terpenoids	Salkowski test	_
Test for phenol	Ferric chloride test	+++
Tests for Phlobatannins		+++
Test for anthraquinones		+++
Tests for resins	Turbidity test	+
Test for fixed oil and fat	Spot test	-

ving 4.735 mg/L and is lower than the WHO unsafe limits indicating the importance of the constituents [46],[30]. Cadmium Cd represents (0.034 mg/L) concentration with the permissible limits of WHO (0.3 mg/L) [47] (Fig. 3a). On the other hand macromolecules like Potassium (K) is the first highest element in *Cistanche tubulosa* stem (20.29mg/L). According to WHO the permissible limit was (28300 mg/L) and it was found that the present value is less the WHO value thus safe for use [48]. Similarly the lowest concentration say 0.775% were represent by calcium represented in the fig 3b.

3. 7. Biological activity

C. tubulosa stem showed significant antibacterial activity by showing a maximum zone of inhibition of 15.1 ± 3.7 against gram-positive bacteria *Staphylococcus aureus* when compared to zone of inhibition of 44.6 of standard positive control Ciprofloxacin respectively. The extract showed minimum zone of inhibition of 12.8 ±4.20 against gram-negative bacteria *Salmonella typhi* when compared to zone of inhibition of 33.6 of standard positive control Ciprofloxacin while showing no inhibitory effect against gram-negative bacteria *Pseudomonas aeruginosa* as shown in (Fig. 4).

The antifungal activity of ethanolic extract of *Cistanche tubulosa* (Schenk) Hook.f. stem was done in. The antifungal activity was tested against *Fusarium oxyfurum, Candida albicans, Penicillium notatum, Aspargilus fumigatus, Aspergillus niger.* The activity showed that the extract possesses antifungal activity at various concentrations and shows minimum inhibition at 50 mg/ml, normal inhibition at 100 mg/ml, and maximum inhibition at 150 mg/ml. As compared to the plant extract, the highest antifungal potential was shown by Flucanozole (antibiotic) and showed varying zones of inhibition (30.6 to 40.9) (Fig 5). The results showed that the highest zone of inhibition was demonstrated by *Candida albicans* (11.0 ± 3.15 mm). The zone of inhibition of the positive control Flucanozole at this fungi was 37.6 mm. The lowest zone of inhibition was demonstrated by *Penicillium notatum* (7.9±6.98 mm). The zone of inhibition of positive control Flucanozole at this fungus was 33.6 mm as shown in Fig. 5).

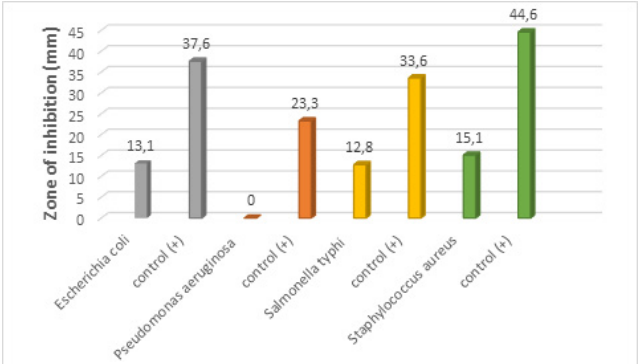

Fig. 4. Antibacterial activity of *Cistanche tubulosa* (Schenk) Hook.f. stem.

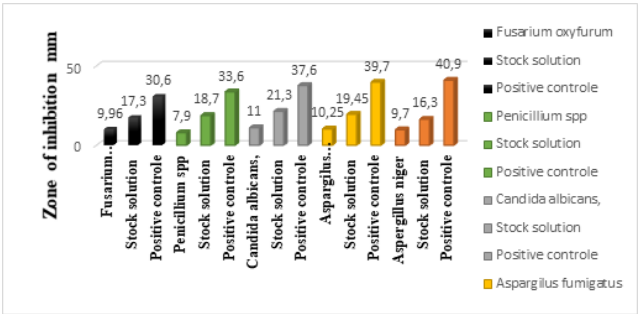

Fig. 5. Antifungal activity of *Cistanche tubulosa* (Schenk) Hook.f. Stem.

4. Discussion

4. 1. Powder Analysis

4. 1. 1. Organoleptic powder drug study

Macroscopic and organoleptic (sensory) evaluations are the main features in the standardization and identification of crude natural drugs and the only parameters that require no involvement of scientific instruments or any expenses. It gives valuable, simplest, quickest and easiest information regarding purity and quality for recognition of adulterants in crude drugs (Khan *et al*., 2017). The study was done with the help of sensory organs including external morphology, colour, odour, taste, and texture of *Cistanche tubulosa* stem and it showed that stem powder was light brown with an acrid taste and unique characteristic odor and smooth texture as shown in (Table 1). Several workers have reported the organoleptic characteristics of the stem of various medicinal plants such as *Detarium microcarpum, Oroxylum indicum,* and hundreds of other medicinal plants regarding the best way in drug evaluation [49],[50].

4. 1. 2. Microscopic study

The microscopic method is one of the most cost-effective and straightforward approaches for accurately identifying crude drugs. These simple yet reliable standards can assist laypersons in using the drug as a home remedy [51]. Microscopic studies or structural details help in the secondary identification of drugs [52]. Microscopic features of the stem of various medicinal plants such as (*Tridax procumbens,* [23] and *Argyreia Pilosa* [53] are reported to be the most distinguishing feature of drug identification. In the powdered microscopy of C*istanche tubulosa* stem there were abundant square shape calcium oxalate crystals, starch grains that were circular to oval in shape, phloem fibres and stone cells, pitted vessels, and trichomes were also observed as shown in (Fig. 7). These anatomical features calcium oxalate, starch grains help to differentiate the species and adulterants.

4. 1. 3. Elemental analysis

All living organisms require macro and microelements within specific ranges. Any disturbance in the levels of these elements can lead to serious and well-known diseases in humans, as the majority of these elements are essential for growth and survival [54]. Medicinal plants contain significant amounts of mineral constituents that are essential for their proper growth and development. Additionally, certain trace elements play a crucial role in the therapeutic properties beneficial to our bodies [55],[56]. The concentrations of all the elements are within the permissible limits set by WHO (1996 and 2005 [57]. The result can assure the usefulness of these elements in the physiological administration of crude drugs since some of the elements were of health benefits and similarly within recommended safe limits [58],[59].

4. 1. 4. Macro- and Micro-elements

Potassium (K) is the most abundant element in the stem of *C. tubulosa*, measured at 20.29 mg/L. According to the World Health Organization (WHO), the permissible limit for potassium is 28,300 mg/L, indicating that this value is significantly lower and thus safe for use. Magnesium (Mg) ranks as the second most abundant element in the stem, with a concentration of 6.731 mg/L, while the WHO permissible limit for magnesium is 68,500 mg/L [48]. Potassium helps in the regulation of water, acid-base balance, regulated blood pressure as well as responsible for nerve actions and the functioning of the muscles [60]. The cofactor, Magnesium (Mg) for many enzymes involved in energy metabolism, helps in the transmission of nerve impulses, detoxification, body temperature regulation, energy production, heart diseases and the development of fit bones and teeth [26],[61]. Other elements like Calcium (Ca) (6.733 mg/L) WHO value (24250 mg/L), and Sodium (Na) (0.77 mg/L respectively show the lower value the WHO limits evidence of the medicinal value of the plants *Cistanche*. Calcium is mostly helpful in bone curing while sodium regulates the osmolality of cells [62] [63]. Microelements like Iron (Fe) play a role in metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport. However, the excessive concentration may also cause tissue damage [64]. In *C. tubulosa* stem, iron is the fourth highest element amount having 4.735 mg/L (WHO for iron was (81.25mg/L), a very small amount (0.215mg/L) (WHO value 1.4462 mg/L) WHO unsafe limits indicating the importance of the constituents [30],[48]. High concentrations of cadmium (Cd) can inhibit growth and induce hepatic and renal impairment. In *C. tubulosa*, the cadmium concentration is measured at 0.034 mg/L, which is well below the WHO permissible limit of 0.3 mg/L [65]. It means that the plant contains a low level of cadmium which is good for use. Other important micro-elements like Lead (Pb), Nickel (Ni), Zinc (Zn), and Cobalt (Co**)** play a key role in growth, genetic expression, and methionine metabolism are represented with prescribed value (0.00mg/L), (0.028 mg/L), (0.104 mg/L) respectively are lower than the WHO limits show the importance of medicinal value of *Cistanche* [66] [58],[65]. *C. tubulosa* has a low amount of cobalt concentration, while there are no regulatory limits by WHO/FAO for Co content in herbal plants as well [67].

4. 2. Fluorescence analysis along with other important feature

When the powder of *C. tubulosa* stem was treated with different reagents it showed different colors in ordinary light, UV 254nm, and UV 366nm which are light brown, brown, olive green, reddish brown, yellowish brown, dark green, dark brown as shown in the (Table 2). The most repeated colors were dark brown, dark green and black. The fluorescence analysis was valuable for the quality control of drugs and valuable in preparing the standards of the quality of the powdered drug and helping identify of practical application of drugs[15],[68]. Several researcher agree with the present finding and have reported the fluorescence analysis of the stem of various medicinal plants *Cicer arietinum, Couroupita guinnensis, Cressa cretica,* [69],[70] and they agree with the present finding. However, the nutritional significance of plants was studied under Proximate and nutritional analysis evaluating the nutritional significance of medicinal plants [71]. The determination of physiochemical parameters is important in the determination of adulterants and improper handling of crude drugs [51] also revealed in the present work (Fig.2). Stem of *Cistanche tubulosa* contains ash contents of (6.3%). The total ash value is a diagnostic purity index and they may be carbonates, phosphates, nitrates, sulphates, chlorides and silicates of various metals, inorganic salts, left

after incineration which is either naturally occurring in drugs or adhering to it or deliberately added to it as a form of adulteration [72],[73]. Working on *Calotropis procera* ash content similar conclusions were obtained by Asoso *et al.,* [74]. Moisture content is an important parameter that needs to be considered during the storage of dried raw herbal material. The moisture content of the drug should be in minimum concentration to avoid bacterial and fungal growth during storage [75]. Stem of *Cistanche tubulosa* contains moisture content (3.23 %). Prevention of diverticulosis gut is mainly regulated by the presence of fiber. A higher concentration of fiber is more valuable than a lower one. *Cistanche tubulosa* contains fiber contents (6.6%) (WHO the permissible value was (35 to 40 %) [76, 77],[78] indicating the importance of plant medicinally.

4. 3. Important contents (Protein, Fate, Carbohydrates (%)

Protein, Fats (lipids) and Carbohydrates are complex biomolecules in the body and act as the primary source of energy [71]. Stem of *Cistanche tubulosa* contains protein content (8.3%), fats content (1.5% and (74.07%) (WHO permissible value was (10 % to 35 %, 35 %, 70 to 77 %) [77]. It was found that the present values were less than the WHO permissible representing that the part is safe to use [79]. Similar results were found on *Talinum triangulare* for protein and carbohydrate contents and their nutritional importance [80].

4. 4. Phytochemical analysis

The initial phytochemical screening of Phytochemicals investigations may be helpful in the designation of the bioactive principles and subsequently may lead to drug discovery and development. Further, these tests facilitate the separation of pharmacologically active chemical compounds and their qualitative and quantitative estimation [2]. Phytochemicals may be primary compounds such as chlorophyll, proteins and common sugars and secondary compounds are terpenoids, alkaloids and phenolic compounds [81]. Flavonoids present in the stem of *Cistanche tubulosa* may have free radical scavenging capacity, antioxidative activity, coronary heart disease prevention, anti-inflammatory, antiviral, anticancer and hepatoprotective activities [82]. Alkaloids help in protein synthesis and act as anti-malarial drugs and cough expectorants [83]. Phenolic compounds had antiaging, anti-apoptosis, anti-atherosclerosis, anti-inflammation, anticarcinogenic, and inhibition of cell proliferation [84]. Tannins can inhibit the growth of microorganisms and act as an antifungal agent at higher concentrations by coagulating the protoplasm of the microorganism. It is also used to allow antibody access in intercellular protein, while the sugar helps in nutritional value [85]. Terpenoids act in antiviral, antibacterial, anti-inflammatory, antimalarial, inhibition of cholesterol synthesis. Glycosides can suppress and soothe irritant dry coughs and act as diuretics [86]. Phlobatannins and Anthraquinones have been reported for their wound-healing properties, anti-inflammatory, analgesic and treatment of metastatic breast cancer along with laxative properties (Ezekiel *et al.,* 2016). Different types of tests were performed to identify these secondary metabolites in the stem of *Cistanche tubulosa* and the results are summarized in (Table 3). The preliminary phytochemical screening conducted on the ethanolic extract of the stem of *Cistanche tubulosa* showed the presence of carbohydrates, proteins, phenols, glycosides, saponins, tannins, flavonoids, phlobatannins, antraquinones, steroidal glycoside, and resins but alkaloids, terpenoids and fixed oil and fats are absent. Many other workers reported phytochemical analysis on the stem of different medicinal plants such as (*Acacia auriculiformis*, Sharma *et al*., (2016); *Andrographis neesiana*, Alagesaboopathi and Sivakumar, (2011); *Mangifera indica,* Somkuwar and Kamble, (2013); *Cassia sieberiana*, Evenamede *et al.,* (2019). The medicinal values of plants lie in some chemical substances that have a definite physiological action on the human body. The presence of some of these secondary metabolites suggests that the plant might be of medicinal importance and supports the bases for some of the ethno uses.

4. 5. Antibacterial activity

Cistanche tubulosa stem showed significant antibacterial activity by showing maximum zone of inhibition 15.1 ± 3.7 against gram-positive bacteria *Staphylococcus aureus* when compared to zone of inhibition of 44.6 of standard positive control Ciprofloxacin respectively. The extract shows minimum zone of inhibition 12.8 ±4.20 against gram-negative bacteria *Salmonella typhi* when compared to zone of inhibition of 33.6 of standard positive control Ciprofloxacin while showing no inhibitory effect against gram-negative bacteria *Pseudomonas aeruginosa* as shown in (Fig. 4). This is in agreement with the previous reports of various medicinal plants *Verbena officinalis*, *Bacopa monnieri*, *Musa acuminata*, [87] [88]. *Salmonella typhi* shows similar results in chloroform extract of *Enicostemma littorale* [89]. Flavonoids and other physiochemical constituents of the plant have antimicrobial properties. The glycosides are used by traditional medicine practitioners in healthcare systems in the treatment of some bacterial infections such as cough, fever, cold and venereal diseases [90].

4. 6. Antifungal activity

The antifungal activity of ethanolic extract of *Cistanche tubulosa* (Schenk) Hook.f. stem was done in (Table 5, Fig. 5). The antifungal activity was tested against *Fusarium oxyfurum, Candida albicans, Penicillium notatum, Aspargilus fumigatus, Aspergillus niger.* The activity showed that the extract possesses antifungal activity at various concentrations and shows minimum inhibition at 50 mg/ml, normal inhibition at 100 mg/ml, and maximum inhibition at 150mg/ml. As compared to the plant extract, the highest antifungal potential was shown by Flucanozole (antibiotic) and showed varying zones of inhibition (30.6 to 40.9) (Table 4). The results showed that the highest zone of inhibition was demonstrated by *Candida albicans* (11.0 ± 3.15 mm). The zone of inhibition of the positive control Flucanozole at this fungi was 37.6 mm. The lowest zone of inhibition was demonstrated by *Penicillium notatum* (7.9±6.98 mm). The zone of inhibition of positive control Flucanozole at this fungus was 33.6 mm as shown (Table 5, Fig. 5). It was concluded from the result that *Candida albicans* was more susceptible than other fungi [91]. Other work revealed that both steroids and carbohydrates of the majority of medicinal plants play a key role in antifungal activity improving the importance of these substances in medicines [92],[93].

5. Conclusion

Macroscopic and microscopic study of plant part will help in correct identification of drugs concluding from the present study along with literature. The extractive values of the drug are useful to evaluate the chemical constituents present in crude drugs and also help in the estimation of specific constituents soluble in particular solvents. Fluorescence analysis of powder drugs with different chemical reagents in visible light and UV light provides the basis for the standardization of drugs. Elemental analysis showed the presence of different metals in the stem part of *Cistanche tubulosa* with variable concentrations. The phytochemical analysis showed that the stem of *Cistanche tubulosa* (Schenk) Hook.f. contained appreciable amounts of phytochemicals both primary and secondary metabolites. The result of antibacterial and antifungal activity showed that *Cistanche tubulosa* possesses antimicrobial activity against plant and human pathogens due to secondary metabolites such as glycosides, saponins, phenols, tannins, and phlobatannins.

Funding

This research was funded by Taif University, Taif, Saudi Arabia, Project No. (TU-DSPP-2024-39).

Acknowledgments

The authors extend their appreciation to Taif University, Saudi Arabia for supporting this work through project number (TU-DSPP-2024-39).

Conflict of interest

All the authors mentioned in the manuscript have no conflict in the research work and compilation.

References

1. Bonati A (1991) Industry and conservation of medicinal plants. In: Proc Int Conf: 21-27 Mar 1988; Chiang Mai, Thailand, 141-145. doi: 10. 1007/s12345-0001-2
2. Prasad M, Srivastava JN, Dantu PK, Ranjan R (2019) Medicinal plants of DEI herbal garden, Dayalbagh: A survey. J Pharm Phytochem 8 (5): 06-22. doi: 10. 22271/jpp. 2019. v8. i5. 1234
3. Debnath M, Malik C, Bisen PS (2006) Micropropagation: a tool for the production of high quality plant-based medicines. Curr Pharm Biotechnol 7 (1): 33-49. doi: 10. 2174/138920106775096183
4. Khan MSA, Ahmad I (2019) Herbal medicine: current trends and future prospects. In: New look to phytomedicine. Elsevier, pp 3-13. doi: 10. 1016/B978-0-12-819123-4. 00001-2
5. Shah A, Sarvat R, Shoaib S, Ayodele A, Nadeem M, Qureshi T, Ishtiaq M, Abbas A (2018) An ethnobotanical survey of medicinal plants used for the treatment of snakebite and scorpion sting among the people of Namal Valley, Mianwali District, Punjab, Pakistan. Appl Ecol Environ Res 16 (1). doi: 10. 15666/aeer/1601_01
6. Aslam Z, Roome T, Razzak A, Aslam SM, Zaidi MB, Kanwal T, Sikandar B, Bertino MF, Rehman K, Shah MR (2022) Investigation of wound healing potential of photo-active curcumin-ZnO-nanoconjugates in excisional wound model. Photodiagn Photodyn Ther 39: 102956. doi: 10. 1016/j. pdpdt. 2022. 102956
7. Patti G, Yanes O, Siuzdak G (2012) Innovation: Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 13: 263-269. doi: 10. 1038/nrm3293
8. Song Y, Song Q, Li J, Zhang N, Zhao Y, Liu X, Jiang Y, Tu P (2016) An integrated strategy to quantitatively differentiate chemome between Cistanche deserticola and C. tubulosa using high performance liquid chromatography–hybrid triple quadrupole-linear ion trap mass spectrometry. J Chromatogr A 1429: 238-247. doi: 10. 1016/j. chroma. 2016. 01. 045
9. Ali SI, Qaiser M (1986) A phytogeographical analysis of the phanerogams of Pakistan and Kashmir. Proc R Soc Edinb B 89: 89-101. doi: 10. 1017/S0370165800012210
10. Morikawa T et al. (2019) A review of biologically active natural products from a desert plant Cistanche tubulosa. Chem Pharm Bull 67 (7): 675-689. doi: 10. 1248/cpb. c18-00738
11. Akhtar K et al. (2019) Prevalence of tick infestation in cows of various Regions of district Karak, Pakistan. J Entomol Zool Stud7 (2): 791-795.
12. Adnan M et al. (2014) Ethnomedicinal and phytochemical review of Pakistani medicinal plants used as antibacterial agents against Escherichia coli. Ann Clin Microbiol Antimicrobials13 (1): 1-18.
13. Zhang W et al. (2016) Extraction, purification, characterization and antioxidant activities of polysaccharides from Cistanche tubulosa. Int J Biol Macromol93: 448-458.
14. Hanani E et al. (2019) Pharmacognostical and Preliminary Phytochemical Evaluation and Flavonoids Content of Paederia foetida Linn. Res J Pharm Phytochem11 (2):81-86.
15. Ishtiaq S et al. (2016) Pharmacognostic studies of aerial parts of Colebrookea oppositifolia Sm. Ann Phytomed5 (2):161-167.
16. Arowosegbe S (2019) Comparative studies on the phytochemical content and proximate composition of different parts of Moringa oleifera plant grown in Ado-Ekiti, Nigeria.
17. Fayaz M et al. (2019) Phytochemical screening and nutritional analysis of some parts of Celosia argentea L. Chem Sci8 (1):12-19.
18. Prakash A et al. (2019) Development and standardization of quality control parameters of different parts of Trianthema portulacastrum L. SN Appl Sci1:1-14.
19. Papalexi E et al. (2018) Single-cell RNA sequencing to explore immune cell heterogeneity. Nat Rev Immunol18 (1):35-45.
20. Dastagir G et al. (2016) Elemental, nutritional, phytochemical and biological evaluation of Hypericum perforatum Linn. Pak J Pharm Sci 29 (2).
21. Sharma N et al. (2017) Pharmacognostical standardization and preliminary phytochemical investigations on Acacia auriculiformis A. Cunn. Ex. Benth stem bark. J Med Plants 5 (1):398-402.
22. Bhutada PR et al. (2016) Solvent assisted extraction of oil from Moringa oleifera Lam. seeds. Ind Crops Prod 82:74-80.
23. Pande J et al. (2018) Pharmacognostic, physicochemical and phytochemical studies of Andrographis echioides Nees. and Tridax procumbens L. leaf and stem. Pharm Innov J 7 (6):303-315.
24. Dini A et al. (1994) Chemical composition of Lepidium meyenii. Food Chem 49 (4):347-349.
25. Kyriakidis NB et al. (2000) Calculation of iodine value from measurements of fatty acid methyl esters of some oils :comparison with the relevant American oil chemists society method. J Am Oil Chem Soc 77 :1235 -1238.
26. Namdev P et al. (2015) Herbal green tea formulation using Withania somnifera stems, Terminalia arjuna bark, Cinnamonbark and Tinospora cordifolia stems and nutritional & phytochemical analysis. J Pharm Phytochem 4 (2):282-291.
27. Rahal A et al. (2014) Proximate and elemental analyses of Tinospora cordifolia stem. Pak J Biol Sci 7 (5):744 -747.
28. Hach CC et al. (1985) A powerful Kjeldahl nitrogen method using peroxymonosulfuric acid. J Agric Food Chem 33 (6):1117 -1123.
29. Njume C et al. (2020) Proximate and phenolic composition of selected native Australian food plants. Int J Food Sci Technol 55 (5):2060 -2079.
30. Ogidi OI et al. (2019) Ethno pharmacologically active compo-

nents of Brassica Juncea (Brown Mustard) seeds. Int J Pharm Res Dev1 (1):9 -13.