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Pharmacognostical with preliminary phytochemical studies of Iraqi Aswagandha (*withania somnifera* L.) plant.

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ABSTRACT

This study was conducted to macroscopical and microscopical investigation with preliminary phytochemical study of As wagandha plant was grown wild under semi-arid condition of Iraq .plant samples were collected from medicinal plants garden of pharmacognosy department and after collection the results of Pharmacognostical study was referred to different macroscopical properties were included a dense, hairy erect grayish tormentors herb or under shrub . The leaves are simple, alternate or sub-opposite, round –oval shape. The flowers are greenish-yellow and found in few flowered clusters in axils. The seeds are many, yellow kidney shape and discoid. The microscopical results were referred to anisocytic stomata, non glandular multi cellular branched trichomes and 22.9% , 31.7% as stomatal indexes for upper and lower surface of leaves respectively . The results of preliminary phytochemical study was referred to ethanolic extract of leaves part was contain flavonoids, alkaloids and coumarin. Ethanolic extract of stems was contain flavonoids, alkaloids and coumarin while ethanolic extract of seeds was contained saponins and phytosterol. The leaves extract by ethyl acetate was contained tannins, saponins, flavonoids, alkaloids and coumarins . Ethyl acetate extract of stems was contained alkaloids and coumarin, while the seed extract by ethyl acetate was contained coumarin and phytosterol.

Keyword: Aswagandha , phytosterol , *withania somnifera* , phytochemical .

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INTRODUCTION

In the last few years , there has been an exponential growth in the field of herbal medicine , and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects [1] . The medicinal plants is a biosynthetic laboratory, not only for chemical compounds but also a mass of the compound from the beginning of time, herbs have been used for healing purposes and most of the worlds people are still using herbs as remedies for various diseases [2] With ania somnifera L. is an important medicinal plant, as small, woody shrub 60-200 cm high belong to solanaceae family , which is described under many common names such as red ginseng , ashogandha [3,4]. The scientific classification of the plant has been presenting in the figure (1) . In ayurvedic and unani system , the leaves of the plant are used for tumor and tubercular glands . Leaves are bitter in taste and used as an antihelminthic [5,6] . The decoction of the root boiled with milk and ghee is recommended for curing sterility in women [7].The roots are also used in constipation, senile debility , rheumatism , general debility , nervous exhaustion , loss of memory, loss of muscular energy and spermatorrhoea [8]. Withanolides that contribute to most of the biological activity of W. somnifera [9]. The chemistry of this plant has been extensively studied and several group of chemical constituents of leaves such as steroidal lactones , alkaloids , tannin etc. [10]. Moe than 12 alkaloids ,40 withanolide containing a glucose molecule at carbon (27) have been isolated and reported from aerial parts . The major chemical constituents of these plants , withanolids are many localized in leaves and their concentration usually ranges from 0.001 to 0.5 % dry weight [11] . Quality and quantity of active compound plants affected by different environmental condition such as type of soil , temperature , light , water supply etc. Iraqi ashogandha plant grown wildly under semi- arid conditions of middle region of Iraq . the aim of this study was conducted to investigate the morphological or macroscopical properties and microscopical properties of plant with preliminary phytochemical study , by using three different types of organic solvent , were sterile water , ethanol 70% and ethyl acetate .

Scientific classification

Kingdom: Plantae
Order: Solanales
Family: Solanaceae
Genus: Withania
Species: somnifera

Fig 1: Withania somnifera

MATERIALS AND METHODS

Collection of Plant samples

Leaves , seeds and stem were collected from the ashogandha plant from medicinal plants garden of college of pharmacy . It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and rinsed with distilled water. The

samples of plant were taken and kept under shade till drying . the plant material was ground in blender and weighed .

Plant extraction

Aqueous Extract

Plant material (100 g) was crushed in sterile water (250 ml) for preparation of aqueous extract. The extract was separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02).

Ethanol Extraction

W. somnifera leaves, seeds and stems (100 g) were ground into fine powder using a stainless-steel grinder, deep in 70 % ethanol (200 mL) for overnight. The ethanol fraction was separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02). The filtered extract was concentrated by a rotary evaporator.

Ethyl acetate Extraction

For preparation of Ethyl acetate extract ground plant samples (100 g) were added in Ethyl acetate and left for overnight at room temperature. The extracts were separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02).

Preliminary phytochemical screening

Phytochemical screening:

The preliminary phytochemical study of ethanolic areal parts extract was carried out by standard methods of phytochemical screening such as mayers , dragendroffs , borntragers test, for alkaloids and glycosides . the foam test , lead acetate test, ferric chloride test, alkaline test [12.13.14] .

Pharmacognostical evaluation:

Macroscopic examination:

Fresh samples of W. somnifera plant were used to study the morphological character of the plant such as shape and margins of leaves, stems and seeds.

Microscopic examination:

Powdered microscopy

Shade dried leaves were finely powdered and examined under microscope. Small quantity of the powder was placed on slide which then added two drops of chloral hydrate and covered with cover slip and examined under microscope. Different cell components were observed and photography was done by using digital camera .

Leaf microscopy:

The two sided of epidermal layer of fresh leaf (in fragments) were mounted in chloral hydrate and observed under a microscope. Determination of the components of leaves (stomata , trichomes and stomatal index) were carried out under microscope . The stomatal index was carried out by using the following equation [15.16].

$$\text{Stomatal index} = \frac{\text{No: of Stomata}}{\text{No: of Stomata + No: of epidermal cells}} \times 100$$

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RESULTS AND DISCUSSIONS

The secondary metabolites compounds considered as active ingredients in plants and there are responsible for different biological activity such as antibacterial , antioxidant , anti-inflammatory ….etc. (40) . In this study the active compounds extracted from different parts of ashogandha plant (leaves , stems , seeds) by different solvents (water, ethyl acetate , ethanol). The screening of ethanol , ethyl acetate and aqueous extract were showed the presence of active constituents in different parts table 1, 2, 3). Terpenoid compounds were not present in all aerial parts in all extracts . Alkaloids absence in seeds extract in all solvents used, while alkaloids and coumarin present in leaves and stems in all solvents extract .The results of preliminary phytochemical study was referred to ethanolic extract of leaves part was contain flavonoids , alkaloids and coumarin. Ethanolic extract of stems was contain flavonoids , alkaloids and coumarin while ethanolic extract of seeds was contained saponins and phytosterol . The leaves extract by ethyl acetate was contained tannins , saponins , flavonoids , alkaloids and coumarins . Ethyl acetate extract of stems was contained alkaloids and coumarin , while the seed extract by ethyl acetate was contained coumarin and phytosterol . Leaves aqueous extract was contained tannins , saponins , flavonoids , alkaloids , coumarin and phytosterol. Stem aqueous extract was contained saponins flavonoids , alkaloids and coumarin , while seeds aqueous extract was contained tannins , saponins , flavonoids , coumarin and phytosterol.

Pharmacognostical evaluation:

The microscopical examination of the leaf showed the presence of anisocytic stomata in which the guard cells are surrounded by three subsidiary cell one of them very smaller than others as shown in figure 2. Non glandular Multi cellular branched trichomes were represented shown in figure 3. Also the microscopic examination was showed about 22.9 % as stomatal index of upper surface while 31.7 % for lower surface of leaves shown in Table 4.

Table 1: Preliminary phytochemical analysis of Withania somnifera leaves .

S.NO	Phytochemicals test	Ethanol Extract	Ethyl acetate Extract	Aqueous Extract
1	Tannins	-	+	+
2	Saponins	-	+	+
3	Flavonoids	+	+	+
4	Terpenoids	-	-	-
5	Alkaloids	+	+	+
6	Coumarin	+	+	+
7	phytosterol	-	-	+

+ = Present, - = Absent

Table 2: Preliminary phytochemical analysis of Withania somnifera stem

S.NO	Phytochemicals test	Ethanol Extract	Ethyl acetate Extract	Aqueous Extract
1	Tannins	-	-	-
2	Saponins	-	-	+
3	Flavonoids	+	-	+
4	Terpenoids	-	-	-
5	Alkaloids	+	+	+
6	Coumarin	+	+	+
7	phytosterol	-	-	-

+ = Present, - = Absent

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Table 3: Preliminary phytochemical analysis of Withania somnifera seeds

S.NO	Phytochemicals test	Ethanol Extract	Ethyl acetate Extract	Aqueous Extract
1	Tannins	-	-	+
2	Saponins	+	-	+
3	Flavonoids	-	-	+
4	Terpenoids	-	-	-
5	Alkaloids	-	-	-
6	Coumarin	-	+	+
7	phytosterol	+	+	+

+ = Present, - = Absent

Table 4: Stomatal index

Upper surface		
No. of stomata	No. of epidermal cell	Stomatal index %
11	37	22.9%
Lower surface		
No. of stomata	No. of epidermal cell	Stomatal index
20	43	31.7%

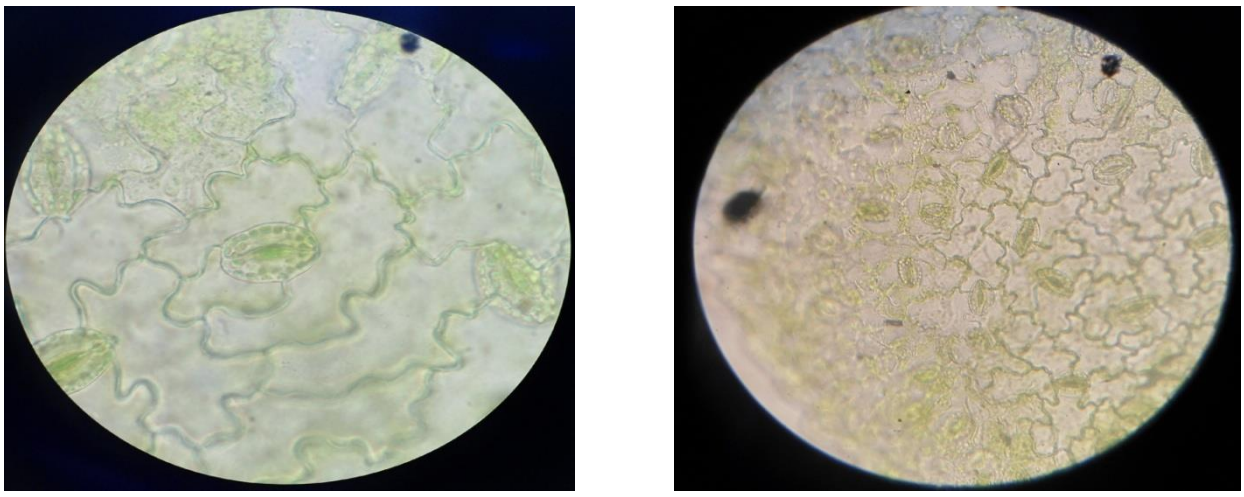

Figure 2: a-Anisocytic stomata 40x b- Anisocytic stomata10x

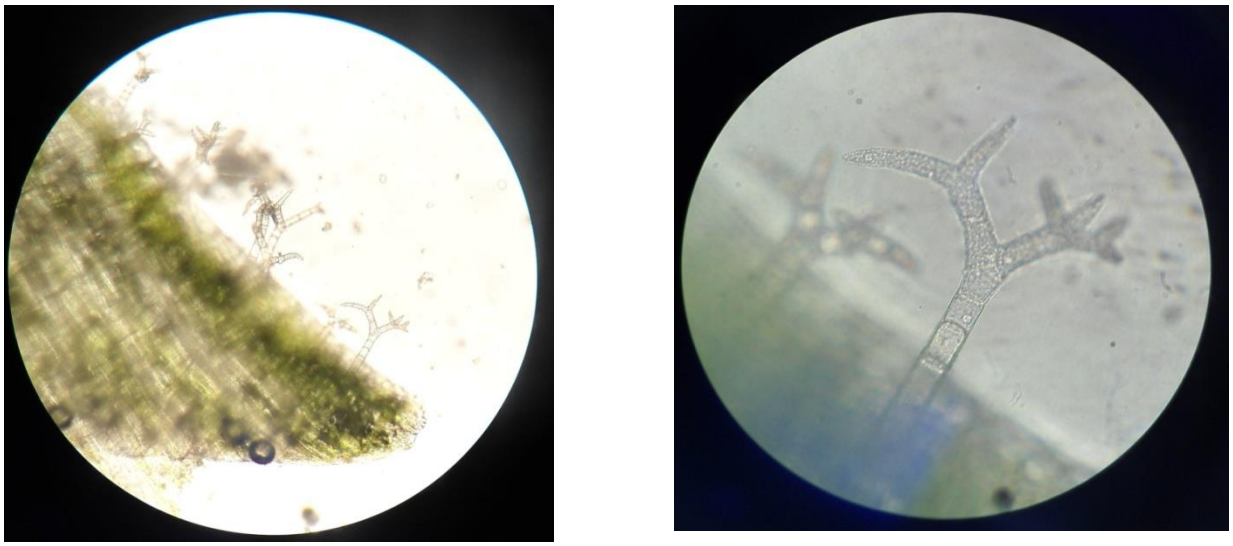

Figure 3: a-Non glandular Multicellular branched trichomes 10x b) 40x

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