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EVALUATION OF NUTRITIVE VALUE, PHYTOCONSTITUENTS, ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF IRAQIAN *WITHANIA SOMNIFERA* L.

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Abstract

This research targeted to estimate the nutritive value, active phytoconstituents, antioxidative and antimicrobial potential of *Withania somnifera* L. Dunal. used in the traditional medicine of Iraq. Primary metabolites of *W. somnifera* were estimated where, the proteins, lipids and nitrogen found to be 19.05, 1.41 and 3.05 gm/100gm dry plant while the fibers, ash and total carbohydrates were found to be 25.61, 20.67 and 28.97 gm/100gm dry plant, respectively. The nutritive value of *W. somnifera* (204.74 calories/gm) provide evidence for using this plant as humans and animals fodder. Aqueous and methanol extracts of *W. somnifera* proved to contain appreciable content of phenolics, flavonoids, tannins, saponins and alkaloids. The extracts expressed good antioxidant activity. Methanol extract expressed antimicrobial potential against many microbes comprising *Bacillus subtilis*, *Staphylococcus aureus*, *Erwinia carotovora* and *Candida albicans* while the aqueous extract did not express any activity. It could be concluded that, *W. somnifera* could be used as fodder due to its nutritive value, as source of antioxidants and antimicrobials so might be included as food supplements and drugs.

Key words: *W. somnifera*, nutritive value, active constituents, antioxidants, antimicrobials.

Introduction

Withania somnifera L. Dunal (family Solanaceae) has many common names like poison gooseberry, ashwagnda, Indian-ginseng or winter-cherry (Chauhan, *et.al*., 2018; Joshi, *et.al*., 2015; Pandey, *et.al*., 2017). It is used in folklore medicine to cure musculoskeletal illnesses like rheumatism and arthritis, as tonic, to improve health and to prohibit athletics, elderly and pregnancy diseases (Halder, *et al*., 2015; Ashok and Shashikant, 2018).

W. somnifera is a dwarf shrub ranged from 35 to 75 centimeters height. It possesses to mentose radial branches with green elliptic leaves with length from 10 to 12 centimeters. The plant has tinny flowers looks like bells. The fruits colors are orange to reddish (Singh, *et al*., 2018).

several bioactive chemicals were recognized in *W. somnifera* like steroids (withaferin and withanolide), saponins and alkaloids and primary metabolites of importance like reducing sugars, starch and potent amino acids (Mahalakshmi, *et al*., 2018; Rastogi and Mehrotra, 1998).

W. somnifera possess anti-inflammatory (Gupta, and Sharma, 2019), anti-cancer (Dutta, *et al*., 2019), antioxidantive (Halliwell, and Gutteridge, 1989; Bagde, and Biswas, 2019) and rejuvenate characteristics (Sachin, *et al*., 2017). It has positive influences on the endocrine, cardiac and nervous system (Lopresti, *et al*., 2019). The research on the cytotoxicity of this plant proved it safety for usage in folklore medicine. (Mahalakshmi, *et al*., 2018).

This research targeted to estimate the nutritive value, phytochemicals and biological potential of *W. somnifera* regarding antioxidant and antimicrobial characteristics.

Materials and methods

Primary metabolites estimation:

Moisture, ash, fiber, lipids, proteins and total carbohydrates contents were determined by AOAC methods (AOAC, 2016).

Lipids were extracted using light petroleum ether by

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Soxhlet. The nitrogen content was estimated using Kjeldahl assay while proteins content was estimated by multiplying the total nitrogen by 6,25. Total sugars were estimated by the method of Masuko *et al.,* (Masuko, *et al*., 2005) meanwhile Miller method was used for determination of reducing sugars (Miller, 1959).

Estimation of nutritive value

The Nutritive value = 4.1x proteins% + 9.2x lipids% + 4.1x total carbohydrates%) and expressed as calories per100 gram dried plant (Burlingame, 2000).

Preparation of the plant extracts

The active ingredients of the leaves of *W. somnifera* were extracted by methanol and 65-70°C water. Five grams of *W. somnifera* leaves were extracted by hundred milliliters of methanol upon shaking for 120 minutes at 200 rotations per minute and other 5 gm were extracted by water at 60 - 65°C for 15 minutes. The obtained filtrates were evaporated to dryness and the dry yield was estimated.

Determination of phytochemicals:

Total phenolics:

Phenolics were estimated according to the method assigned by Lin and Tang, (Lin, and Tang, 2007) and calculated as gram gallate equivalent/100-grams dry material.

Total flavonoids:

Flavonoids were estimated according to the method assigned by Chang *et al*., (Chang, *et al*., 2002) and calculated as gram quercetin equivalent/100-grams dry material.

Total tannins:

Tannins were determined according to Vanillin hydrochloride method (Sadasivam, and Manickam, 1996) and were calculated as gram gallate equivalent/100-grams dry material.

Total alkaloids:

Alkaloids were determined according to the method assigned by Singh *et al*., (Singh, *et al*., 2004).

Determination of antioxidant activity:

Diphenyl picryl hydrazil (DPPH) method:

The antioxidant activity determined by "DPPH°" method of Liyana- Pathirana and Shahidi, (Liyana-Pathirana, and Shahidi, 2005). The concentration of antioxidant capable to diminish the used DPPHÿ concentration by 50% ($IC_{50\%}$) was determined. Vitamin C was used as standard compound.

Ferric Reducing Antioxidant Power (FRAP) method:

Antioxidant potential was estimated using FRAP assay assigned by Benzie and Strain (Benzie, and Strain, 1996), and determined as the concentration of antioxidant possessing a ferric reducing capability equal to 1 mM $FeSO_4.7H_2O$.

Determination of the antimicrobial potential:

Disc diffusion method:

The antimicrobial potential was estimated using disc diffusion method (Murray, *et al*., 2005).

Pathogens used:

Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Erwinia carotovora, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia and *Candida albicans*.

Results

Phytochemical constituents

Table 1 introduces the primary metabolites in *W. somnifera* leaves. There was appreciable levels of moisture, crude lipids and fibers, proteins, reducing and non-reducing sugars, ash, total carbohydrates and total nitrogen. The nutritive value estimated to be 204.75 cal/ 100 gm dry weight that could act as good fodder.

Quantification of active secondary constituents of *W. somnifera* indicated that the extracts contain remarkable content of phenolics, flavonoids, tannins and alkaloids as illustrated in table 2.

Flavonoids and tannins were higher in methanol than in aqueous extract meanwhile alkaloids and phenolics were higher in that of aqueous than methanol. The

Table 1: Primary metabolites in the leaves of *W. somnifera*.

Measure parameters %	Value
Moisture	7.3
Ash	20.67
Proteins	19.05
Lipids	1.41
Fibers	25.61
Soluble sugars	8.73
Total carbohydrate	28.97
Nutritional value cal/100 gm dry plant	204.75

Table 2: Secondary constituents in *W. somnifera*.

Secondary constituents	Water	Methanol
Phenolics%	1.74	1.31
Flavonoids%	0.25	0.38
Tannins%	0.19	0.26
flavonoids/ phenolics Ratio	0.14	0.28
Alkaloids%	0.71	0.33

phenolics found to be 1.31 and 1.74 gm/100 gm dry plant in methanolic and aqueous extracts, respectively. Alkaloids found to follow phenolics with values of 0.34 and 0.71 gm/100 gm dry plant in methanolic and aqueous extracts, respectively. Flavonoids were found to be 0.38 and 0.25 gm/100 gm dry plant in methanol and aqueous extract, while tannins showed the lowest values of 0.26 and 0.19 gm / 100 gm dry plant in methanolic and aqueous extracts, respectively.

Determination of the antioxidant activity:

There is inverse relationship between the antioxidant activity and IC_{50} (Bouaziz, *et al*., 2005). The results in table 3 illustrated that *W. somnifera* aqueous extract (0.09 mg/ml) was higher than the methanol (0.18 mg/ml), that were comparable to ascorbic acid. The results obtained by FRAP assay agree with those obtained using $DPPH^0$ assay.

Determination of the antimicrobial activity of *W. somnifera*

The antimicrobial activity was determined by measuring inhibition zones arise against many pathogens. Methanol extract showed broad antimicrobial activity against 50% of the tested pathogens including *C. albicans, E. carotovora, S. aureus and B. subtilis*. The aqueous extract expressed no activity against any of the tested microbes as in table 4.

Discussion

This research targeted to estimate the nutritive value of *W. somnifera* and to study the phytoconstituents in its aqueous and methanolic extracts that may play role in their antioxidant and antimicrobial activity.

Primary metabolites play a crucial role in plants' growth and reproduction. They are considered as enhancers for several medicinally bioactive secondary metabolites (Moure, *et al*., 2001). The nutritive value of plants plays a crucial role as human and animal fodder (Pagare, *et al*., 2015).

The results showed that the studied extracts are rich in active antioxidant compounds, possess high antioxidant activity in comparison with that of vitamin C and that may be due to the flavonoids that are well known antioxidants. antioxidant and antimicrobial activity of *W. somnifera* methanol extract was higher than aqueous extract and this might be attributed to presence of phytoconstituents like phenolics, terpenes, flavonoids (Takaoa, *et al*., 2011; Cowan, 1999) and alkaloids (Aslam, *et al*., 2016).

In conclusion, *W. somnifera* could be used as a source of pharmaceuticals, fodders, nutritive supplements, antioxidants and antimicrobials. These phytochemicals justify the folkloric medicinal usages of this plant.

Table 3: Antioxidant activity of *W. somnifera*.

Method	FRAP assay gram dry extract / $mMFe^{+2}$		DPPH assay $IC_{50\%}$ (mg/ml)	
Extracts	Aqueous	Methanol	Aqueous	Methanol
	693.74	890.36	0.09	0.18
Vitamin C	-		0.02	

Table 4: Antimicrobial activity of *W. somnifera* extracts.

Microbes	Methanol extract	Aqueous extract
C. albicans	12	-
E. carotovora	11	-
P. aeruginosa	-	_
S. aureus	12	-
K. pneumonia	-	_
P. vulgaris	-	_
B. subtilis	11	-

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