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NUTRITIONAL, PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF *ARTEMISIA HERBA-ALBA* (ASSO.)

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Abstract

This study estimated the various components of the aerial parts of *Artemisia herba-alba* belonging to family Asteraceae. The components extracted from the plant were elucidated by GC/MS spectroscopic analysis. The data revealed that the extract contains 2-methyl-pentane (0.09%), borneol (0.10%), Camphor (97.61%), caryophyllene oxide (0.10%), t-cadinol (0.09%), preg-4-en-3-one, 12,17-dihydroxy-17-cyano- (0.12%), androstane-3,17-dione (1.74%), cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy pivalate (0.02%) and geranyl isovalerate (0.01%). The nutritional value, phytochemicals, antioxidant and antimicrobial activity were studied using several assays following standard procedures. The nutritional results indicated that aerial parts of *A. herba-alba* are furious source of dietary fiber, carbohydrates, proteins and lipids. The results indicated that the prepared water and methanol extracts expressed high antioxidant scavenging activity. The extracts expressed antimicrobial activity against several pathogenic bacterial strains in addition to one fungal strain. Conclusively, the dried aerial parts of *A. herba-alba* are a good source of healthy constituents that could be used therapeutically and nutritionally.

Key words: *A. herba-alba*, nutritional, GC/MS analysis, phytochemica, antioxidant and antimicrobial activities.

Introduction

Some medicinal plants are utilized in folklore medicine to treat and provide nutrition to humans (Venkataswamy *et al*., 2010). A large sector of people in developing countries continued to rely on folklore medicine (WHO, 2002).

Several studies have demonstrated that medicinal plants are the core of many bioactive phytochemicals that possess the antimicrobial potential and have the ability to protect the human body from stress arises due to free radicals that might cause heart and neurodegenerative disorders, joints inflammation, cancer and several malfunctions. On this basis, it is essential to identify phtotherapies for the treatment of cancer and with antimicrobial potential (Zheng *et al.*, 2008, Ghasemzadeh *et al.*, 2010).

Artemisia is a very valuable genus of Asteraceae that contains about five hundred species. It is a cosmopolitan and distributed in the northern half of the hemisphere (Oberprieler, 2001, Valles and Garnatge, 2005).

Artemisia herba-alba Asso (Arabic name: chih), family "Asteraseae" is an important plant that is used in folk medicine due to its medicinal value and in giving flavor to coffee and tea (Bezza *et al*., 2010). It is known as "wormwood" that is distributed the Middle East countries like Iraq and in Northern Africa. This plant is used in treating stomach and hepatic disorders, also used as anticancer, antispasmodic, antidiabetic, antimicrobial and antioxidant (Bezza *et al.*, 2010, Mighri *et al.*, 2010, Goudjil *et al.*, 2015).

Studies on *A. herba-alba* revealed that this plant is furious with many phytochemicals like flavonoids phenolics, volatile and essential oils and is known for its antioxidant activity (Dif *et al.*, 2016).

Accordingly, this study estimated the chemical structure of the extracted components by GC/MS spectral analysis and evaluates of the nutritional value, phytochemical components, antioxidant and antimicrobial activity of the medicinally valuable *A. herba alba*.

Materials and Methods

Plant material

The aerial parts of *Artemisia herba-alba* were collected in cool and dry containers, air-dried in shade

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for 21 days, grinded and stored for analysis.

Gas chromatography (GC)/EIMS analysis

The analysis of gas chromatographic analysis (GC/EIMS) was performed on Agilent Technologies 7890A GC System (5975C inert MSD with Triple-Axis Detector) equipped with an HP-5MS capillary column coated with 5% phenyl methyl silox (30 m × 250 µm, coating thickness 0.25 µm). Helium at a flow-rate 1 ml/minute and a split ratio of 1:20 was utilized as a gas carrier. 2 µL of the extract was injected. At 40°C "temperature program" for 5 minutes, rising at 3°C/minutes to 200°C and held for 1 minute, rising at 15°C/minutes to 280°C and held for 10 minutes. The injector and detector were apprehended at 250 and 300°C, consistently. The retention times of the investigated components were compared with those of the conveyed samples and their linear retention indices were compared relative to the *n*-hydrocarbons series and computer matching against commercial (NIST11.L and demo.1).

Determination of nutritive value

Crude lipids, crude fiber, crude proteins, ash, moisture and carbohydrates were estimated according to the methods of AOAC (AOAC, 2016). The total sugars contents were determined according to the method of Masuko and co-workers (2005) while the reducing sugar was estimated using the modified assay of Miller (1959).

The nutritive value was finally determined using the following formula

(The nutritive value = 4× protein% + 9× fat% + 4× total carbohydrates%). The energy produced expressed as kcal/100 gram dried plant material taking into consideration that 1gram protein = 4.1 kcal, 1 gram carbohydrates = 4.1 kcal and 1gram lipids = 9.2 kcal (AOAC, 2016).

Extraction

Plant water extract was prepared by 10 grams of the dried aerial parts of *A. hrba-alba* was extracted upon shaking for 30 minutes at 70°C using 150 ml water then filter, while anther 10 grams of the plant were extracted using methanol upon shaking for two hours, then the obtained extracts were evaporated using rotary evaporator.

The active secondary metabolites present in the prepared extracts in addition to their antioxidant and antimicrobial activity of the prepared extracts were determined.

Total phenolics were estimated by the modified Folin Ciocalteu method (Wolfe *et al.*, 2003) and expressed as gm gallic acid equivalent/100 grams dried aerial parts.

Total flavonoids were estimated by aluminum chloride method (Zhishen *et al.*, 1999) and expressed as gm catechin equivalent/100 grams dried aerial parts.

Total Tannins were estimated by Vanillin hydrochloride method (Sadasivam and Manickam, 2008) and expressed as gm gallic acid equivalent/100 gram dried aerial parts.

Total Alkaloids were estimated by the method adopted by Harborne (1999) and expressed as a percentage of the dried aerial parts.

Total Saponins were estimated by the method adopted by Obadoni and Ochuko (2001) and expressed as a percentage of the dried plant.

Screening of antioxidant scavenging activity

Diphenyl picryl hydrazil "DPPH" assay: The antioxidant effect was determined by the assay adopted by Liyana-Pathirana and Shahidi (2005) and the scavenging activity was estimated as the concentration of the plant extracts at which 50% of the DPPH free radicals scavenged ($IC_{50\%}$) using ascorbic acid as reference for comparison.

Antioxidant capacity of plant extracts determined by ($ABTS^{+}$) cation radical assay

ABTS (2,2'-azino-*bis*-3-ethyl benzothiazoline-6-sulfonic acid) assay was adopted by Re *et al.,* (1999) and the used standard was ascorbic acid.

Antimicrobial activity

Microbial susceptibility test: The antimicrobial potential of *A. herba-alba* extracts was estimated using filter paper disc method (Murray *et al*., 1998). Four-gram positive bacteria *(Bacillus subtilius, Staphylococcus aureus*, *Staphylococcus epidermis, Streptococcus pyogenes),* 4 Gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Erwinia Carotovora)* and only one fungal strain *(Candida albicans)* were used.

Table 1: Proximate composition and nutritional value of *A. herba alba.*

Assessments	Compositions
Moisture %	10.69
Crude Ash %	5.90
Crude Protein %	13.20
Crude fat %	1.83
Crude Fiber %	30.80
Reducing sugars %	21.19
Total sugars %	47.27
Non reducing sugars %	26.07
Total carbohydrate %	48.29
Total Nitrogen %	2.73
Nutritive value kcal/100 g dry weight	268.58

Table 2: The phytochemical constituents in *A. herba alba*.

Secondary metabolites	Water Extract	Methanol Extract
Phenolics (gm gallic acid equivalent/100 gm dried plant)	2.09	2.180
Flavonoids (gm catechin equivalent/100 gm dried plant)	0.322	0.305
Tannins (gm gallic acid equivalent / 100 gm dried plant material)	0.076	0.024
Alkaloids %	2.10	3.20
Saponins %	1.25	1.42

Results and Discussion

Proximate primary metabolites composition

The primary metabolites are considered important for plants to grow, develop and reproduce and for humans as a nutritional supplement and for animals as fodder. They are the core for the production of several active metabolites of pharmacological activity (Pagare *et al.*, 2015, Ncube and Staden, 2015).

The proximate composition of aerial parts of *A. herba-alba* is presented in table 1. The results indicated that the aerial parts of *A. herba-alba* contain higher levels of crude fiber, crude ash, crude protein, crude fat, total carbohydrates and total nitrogen. The obtained results illustrated that this plant comprises considerable levels of nutritive components where the nutritional value of *A. herba-alba* is 268.58 kcal/100 g dried aerial parts.

Secondary metabolites

The environmental conditions impact stress are precursors for the production of variable levels of secondary metabolites in plants to defend themselves (Tsao, 2010, Ramamoorthy and Bono, 2007). The dry habitat of *A. herba-alba* is a stimulator for it to synthesize different types of secondary metabolites such as alkaloids, phenolics, tannins, flavonoids, saponins and many other metabolites that possess protective and therapeutic features (Table 2).

Table 3: The elucidated chemical constituents of the extracted *Artemisia herba-alba* Asso.

Entry	Chemical name	Classification	Retention time (min)	Molecular Weight	Molecular formula	Composition %
1	Pentane, 2-methyl-	Alkane	5.18	86.18	C_6H_{14}	0.09
2	borneol	monoterpene derivative	5.69	154.25	$C_{10}H_{18}O$	0.10
3	Camphor	Phenol that is a natural monoterpene derivative	6.77	152.22	$C_{10}H_{16}O$	97.61
4	Caryophyllene oxide	Bicyclic sesquiterpene and a metabolite of β-caryophyllene	8.55	220.36	$C_{15}H_{24}O$	0.10
5	τ-Cadinol	Cadinane sesquiterpenoid	8.85	222.37	$C_{15}H_{26}O$	0.09
6	Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-	Cholesterol derivative	9.75	400.69	$C_{28}H_{48}O$	0.03
7	Preg-4-en-3-one, 12,17-dihydroxy-20-nitrilo- (Preg-4-en-3-one, 12,17-dihydroxy-17-cyano-)	Steroid	10.32	329.44	$C_{20}H_{27}NO_3$	0.12
8	1-(3-hydroxy-3-methylpent-4-en-1-yl)-2,5,5,8a-tetramethyldecahydro naphthalen-2-ol(Scareol)	Terpenoid	10.85	308.51	$C_{20}H_{36}O_2$	0.04
9	Retinyl acetate (retinol acetate)	Vitamin A acetate	11.48	328.50	$C_{22}H_{32}O_2$	0.03
10	Androstane-3,17-dione (5α-androstanedione or as 5α-androstane-3,17-dione)	Steroid	12.60	288.43	$C_{19}H_{28}O_2$	1.74
11	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-, pivalate	Steroid	12.76	498.79	$C_{33}H_{54}O_3$	0.02
12	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	Branched alkane	14.29	366.72	$C_{29}H_{60}$	0.01
13	Geranyl isovalerate (*trans*-3,7-Dimethyl-2,6-octadienyl isopentanoate)	Terpenoid ester	14.98	238.37	$C_{15}H_{26}O_2$	0.01
	Σ					Σ = 99.99

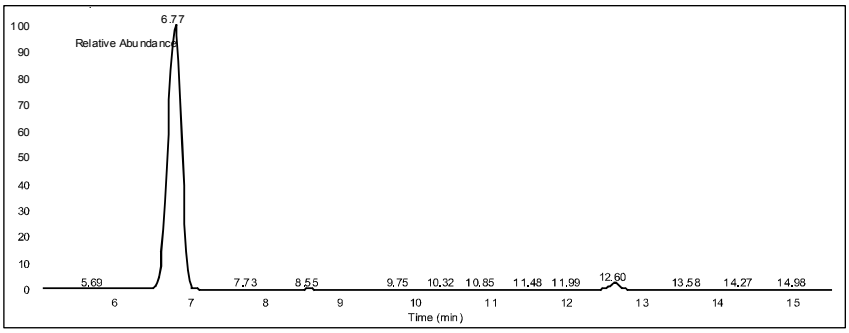

Fig.1: Chromatogram of *Artemisia herba-alba* Asso extracts by GC-MS.

The secondary metabolites composition in aqueous and methanolic extracts of *A. herba-alba* are presented in table 2. The total phenolics, flavonoids and tannins contents were higher in water extracts than methanol extracts while alkaloids and saponins content was higher in methanol extract than that of water.

Chemical composition of the essential oils and fatty content

According to the GC/MS spectroscopic analysis, thirteen compounds in a total composition % (99.99%) were identified from the aerial parts of *Artemisia herba-alba* (Table 3). The data revealed that the extract contains 2-methyl-pentane (0.09%), borneol (0.10%), Camphor (97.61%), caryophyllene oxide (0.10%), -cadinol (0.08%), (preg-4-en-3-one, 12,17-dihydroxy-17-cyano-) (0.12%), androstane-3, 17-dione (1.74%), cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate (0.02%) and geranyl isovalerate (0.01%) (Table 3 and Fig. 1).

Several types of researches reported the extraction of the components from the aerial parts of *Artemisia herba-alba*. Camphor was the main component identified. It was reported in the literature that camphor extracted from different varieties of *Artemisia* using green solvents (Mohamed *et al.*, 2010). The pharmacological and therapeutic potentials of Camphor were recently investigated (Hamidpour *et al.,* 2019). On the other hand, the chemical and pharmacological properties of caryophyllene oxide were conveyed and its anticancer and analgesic properties were reported (Fidyt *et al.,* 2016, Habtemariam, 2019). Also, the extracted τ-cadinol were conveyed to have several pharmacological activities (Occhipinti *et al.,* 2013). Additionally, the aromatase inhibitory activity and antifungal activity of androsta-1,4-diene-3,17-dione against *Aspergillus brasiliensis* were reported (Hosseinabadi *et al.,* 2014, Pokhrel and Ma, 2011). Several types of researches were reported the chromatographic applications of the extracted pivalate (Hashem, 2016, Haffenden and Lawson, https://www.sciencedirect.com/science/article/abs/pii/0022190267800988 - !1967). Also the importance of geranyl isovalerate has approved (Ferraz *et al.,* 2015).

Evaluation of the antioxidant scavenging activity

The antioxidant activity of the studied *A. herba-alba* extracts was estimated by DPPH$^{\bullet}$ and ABTS^{+} methods. Research on plants of medicinal importance found to possess antioxidant activity that was attributed mainly to their phenolics and flavonoids. The highest antioxidant activity of these compounds is due to the hydroxyls present in their structure that are responsible for the free radicals scavenging activity of such plants (Hernández Zarate *et al.*, 2018).

Regarding the DPPH assay, IC_{50} (the concentration of an antioxidant needed to decrease the initial DPPH$^{\bullet}$ radical concentration by 50%) is a parameter used for evaluating the antioxidant scavenging activity. There inverse relationship between IC_{50} and the antioxidant activity (Sanchez Moreno *et al.*, 1998). The data in table 4, showed that the methanolic extract of *A. herba-alba* was higher in its free radical scavenging activity (0.06 mg/ml) than the aqueous extract (0.081 mg/ml). Both extracts expressed higher antioxidant activity that was

Table 4: DPPH scavenging effect of *A. herba-alba* extracts (mg/ml).

Samples	IC_{50} values
Water extract	0.081
Methanol extract	0.060
Ascorbic acid	0.023

Table 5: % Inhibition of the extracts measured by the ABTS assay.

Samples	% Inhibition
Water extract	69.59%
Methanol extract	74.52%
Ascorbic acid	91.45%

Table 6: The antimicrobial activity of the *A. herba-alba* extracts.

Microorganisms	Inhibition zones (mm) of plant extr acts	
	Methanol	Water
Candida albicans	10	10
Erwinia Carotovora	10	-
Pseudomonas aeruginosa	-	-
Staphylococcus aureus	11	-
Klebsiella pneumonia	-	-
Proteus vulgaris	-	-
Streptococcus pyogenes	10	-
Staphylococcus epidermis	12	-
Bacillus subtilius	9	-

comparable to that of ascorbic acid (0.023 mg/ml).

The $ABTS^+$ method was used for evaluating the hydrophilic and lipophilic antioxidants in the studied extracts by estimating the inhibition% of absorbance originated due to decolorization. As illustrated in table 5, the methanolic extract was higher in its activity (74.52%) than the aqueous extract (69.59 %) but with values lower than that of ascorbic acid (91.45%). The results of the DPPH assay agree with those obtained from ABTS assays. The results obtained agree with the fact that there is a direct relation between the increase of total phenolics and total flavonoids and the higher antioxidant activity of the tested extracts.

Evaluation of the antimicrobial activity of *A. herba-alba* (disc diffusion assay)

Antimicrobial drugs of plant origin that are active against antibiotic-resistant microorganisms are the motive for searching for natural products that have antimicrobial activity (Farjana *et al.*, 2014). Many reports are available on the antimicrobial activity of extracts from medicinal plants (Joe *et al.*, 2009, Babotã *et al*., 2018). Phenolics, flavonoids, terpenoids, alkaloids, saponins, coumarins and sterols that were identified in *Artemisia* species have reported possessing antimalarial, antiviral, antibacterial, antifungal and antitumor activity (Tan *et al*., 1998, Rabe *et al.*, 2015).

The antimicrobial potential of aqueous and methanolic extracts of *A. herba-alba* were determined on the basis of their zone of inhibition against many pathogenic strains and the results expressed as a zone of inhibition. The results illustrated that the methanolic extracts showed broad antimicrobial spectrum than water extracts as presented in table 6. The methanolic extract of *A. herba-alba* showed inhibitory activity against 55.56% while that of water extract showed inhibitory activity against only 11.11% of the tested pathogens.

Conclusively, it should be noted that although the extracts of the air-dried aerial parts of *A. herba-alba* comprise several bioactive components with pronounced antioxidant and antimicrobial activities in addition to its excellent nutritional value, more studies are needed to be done regarding this plant in order to isolate and elucidate the active components in order to maximize the nutritional and medicinal value of this plant.

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