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VOLATILES COMPOSITION, PHYSICOCHEMICAL PROPERTIES, KINETIC STUDY AND ANTIOXIDANT POTENTIAL OF ENDEMIC *ARTEMISIA* (*ARTEMISIA JUDAICA* L.) ESSENTIAL OIL

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Abstract:

The present work was conducted to investigate the yield, chemical composition, kinetic study, antioxidant and physicochemical properties of essential oil isolated from the aerial parts of Artemisia judaica harvested from Jazan region of Saudi Arabia. The hydro-distilled essential oil yield was found to be 1.08%. GC-MS analysis confirmed 21 chemical components, representing 98% of the total oil composition. As far as GC-MS volatiles profiling is concerned, (+)-davanone (1) was detected to be the principal component (34.32%) followed by camphor (21.61%). A considerable amount (7.7%) of a blue color imparting compound, chamazulene , was also detected. FT-IR spectrum of the oil also and validated the chemical components identified. Extraction process of A. judaica essential oil was further elucidated through kinetics study, which showed that antioxidant activity was affected in time and concentration dependent manner following second order kinetics. Further, physicochemical properties such as specific gravity, refractive index, acid value, peroxide value and saponification value of the oil were tested and discussed. The findings of this study revealed that endemic species of Artemisia has significant potential for nutra-pharmacetical applications.

Key Words: *Artemisia judaica, GC-MS, Characterization, Kinetic study, Physicochemical properties, Antioxidant, Essential oils*

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INTRODUCTION:

The genus *Artemisia* includes several annual or perennial herbs naturalized in temperate northern regions [1-4]. In particular, *Artemisia* species are grown in temperate climates of both hemispheres, from dry or semiarid to coastal areas [1,5]. The species of this genus have been widely explored as high-valued plants due to their potential uses as folk medicine and food flavoring agent in varieties of cuisines. They exhibit multiple biological effects such as sedative, vermifuge stomachic, diarrheic, antiseptic and antispasmodic [5]. *Artemisia judaica* L., a member of the *Asteraceae* family, is distributed globally including in Saudi Arabia, Egypt, Algeria, Palastine and Jordan [1]. *Artemisia judaica* (*A. judaica*) has been reported to be used in the treatment of heart disease, diabetes [6], gastro-intestinal disorders [7], coronary artery thrombosis and myocardial infarction [8]. From the past few decades, there is increasing attention of plant scientists in the evaluation of essential oils from *Artemisia* species as antioxidant [9], anti-inflammatory, analgesic, anti-pyretic [10], antimicrobial[11] agents. However, a comprehensive study on *Artemisia* essential oil (AEO) suggested that there was a considerable variation in biochemical profiles and yield of oil from different agro-climatic regions [12]. Therefore, based on chemo-taxonomical study it was found that *Artemisia* chemotype essential oils contained varied chemical composition [12]. For example, there is high contents of piperitone and camphor detected in AEO, however Davanone, Germacrene D, *trans*-anethole, Chamazulene, *trans* –ocimine and β-thujone were also present in variable amounts [12].

The Kingdom of Saudi Arabia mainly comprises of arid and desert land mass contributing major part of Arabian peninsula with diverse ecosystems and biodiversity [4,13]. Such a vast agro-ecological diversity allowed growth of wide array of medicinal plants with potential for bioprospecting [14]. In Saudi Arabia, *A. judaica*is is widely grown in Hail region at the northern central part of the Kingdom [15,16], and in the south-western part as well. This herb is locally used as fragrant in southern areas and is known by name shih [17,18], and Khatour or boithiran in Jazan. Currently, there is greater interest of researchers for the identification of chemical constituents and pharmacological properties of herbs essential oils [1,2]. The present work is designed to explore the yield, chemical composition, physicochemical and antioxidant properties of hydro-distilled essential oil from aerial parts of *A. judaica* populations harvested in the Jazan region of Saudi Arabia.

MATERIALS AND METHODS:

Material Collection

The aerial parts were collected from fully mature *Artemisia judaica* L. plants grown in southwestern regions of Saudi Arabia namely Jazan, during 2017. The specimens were further identified and authenticated by a taxonomist at College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-kharj, Saudi Arabia.

Isolation of Artemisia essential oil

The shade-dried *A. judaica* plant material was crushed in a coffee grinder (90 mesh). The crushed material was subjected to hydro-distillation in a Clevenger apparatus (Gulf Scientific Glass Industry, Manama, Bahrain) for 4 h. The received oil was dried over anhydrous sodium sulfate (Na_2SO_4, 99%) in a desiccator, filtered and preserved at -4°C for further experimental work.

GC-MS characterization of Artemisia judaica essential oil (AEO)

GC-MS analysis of AEO was done by using QP 2010/Ultra mass spectrophotometer (Shimadzu, Tokyo, Japan) coupled with AOC. 20i Auto injector. The oil volatile compounds were analyzed/separated using a fused silica capillary column Rtx-5 MS (30 m × 0.25 mm; 0.25 µm) (RESTEK, Bellefonte, PA, USA). Using a micro syringe, a 1.0-µL AEO diluted with methanol (1:4 v/v) was injected into the column via auto injector operated at 290 ºC. The separated volatile chemical constituents were identified and authenticated by comparing their mass spectral (MS) data with those already available in NIST 05 Mass Spectral Library and by co-injecting pure standards [19,20].

FT-IR analysis of AEO

For FT-IR analysis of AEO, a Thermo scientific iD5 ATR diamond Nicolet iS 5 FT-IR Spectrometer was used. The data spacing was 0.482 cm^{-1} with single beam having OMINIC software.

Total phenolics compounds (TPC) and DPPH° radical scavenging activity

Antioxidant activity *AEO* was assessed by determining total phenolic compounds (TPC) and 2,2´-diphenyl-1-picrylhydrazyl stable free radicals (DPPH°) scavenging ability. Folin Ciocalteu reagent (FCR) based colorimetric assay was performed for TPC [21]. The amount of TP was calculated using the standard calibration curve (R^2= 0.997) calculated by running a series of the standard solution of Gallic

acid (GA). The results were presented as mg GAE /100g of tested essential oil. The antioxidant activity of AEO was also evaluated on the basis of its ability to scavenge purple colored 2,2´-diphenyl-1-picrylhydrazyl free radicals (DPPH°). The assay was performed by a standard method as reported earlier [20].

Determination of physicochemical properties of AEO

Physicochemical properties such as specific gravity (25 C^0), acid value, peroxide value and saponification value of AEO were determined by various standard methods reported in the literature [22, 23]. Whereas, the refractive index (25 C^0), of the oil was measured using Automatic Refractrometer (Rudolph, J-257, NJ, USA).

Percent Yield and Chemical Composition of Hydro-distilled Artemisia judaica Essential Oil

The hydro-distilled essential oil yield from *Artemisia judaica* aerial parts was found to be as high as 1.08%. *Artemisia* essential oil was analyzed by GC-MS (Fig. 1).A total of 21 chemical components, representing 98% of the overall oil composition were identified. Davanone was detected to be the principal component (34.3%) in the oil followed by camphor (21.6%). A considerable amount (7.7%) of blue color imparting compound, chamazulene, was also detected (Fig 2). The chemical composition of other *Artemisia species* showed quite variable results. Majority of *Artemisia* species furnish essential oils containing piperitone and camphor as major components, while davanone and camphor were found to be the main compounds in the *Artemisia judaica* essential oil analyzed in the present experiments (Table 1) .

RESULTS AND DISCUSSION:

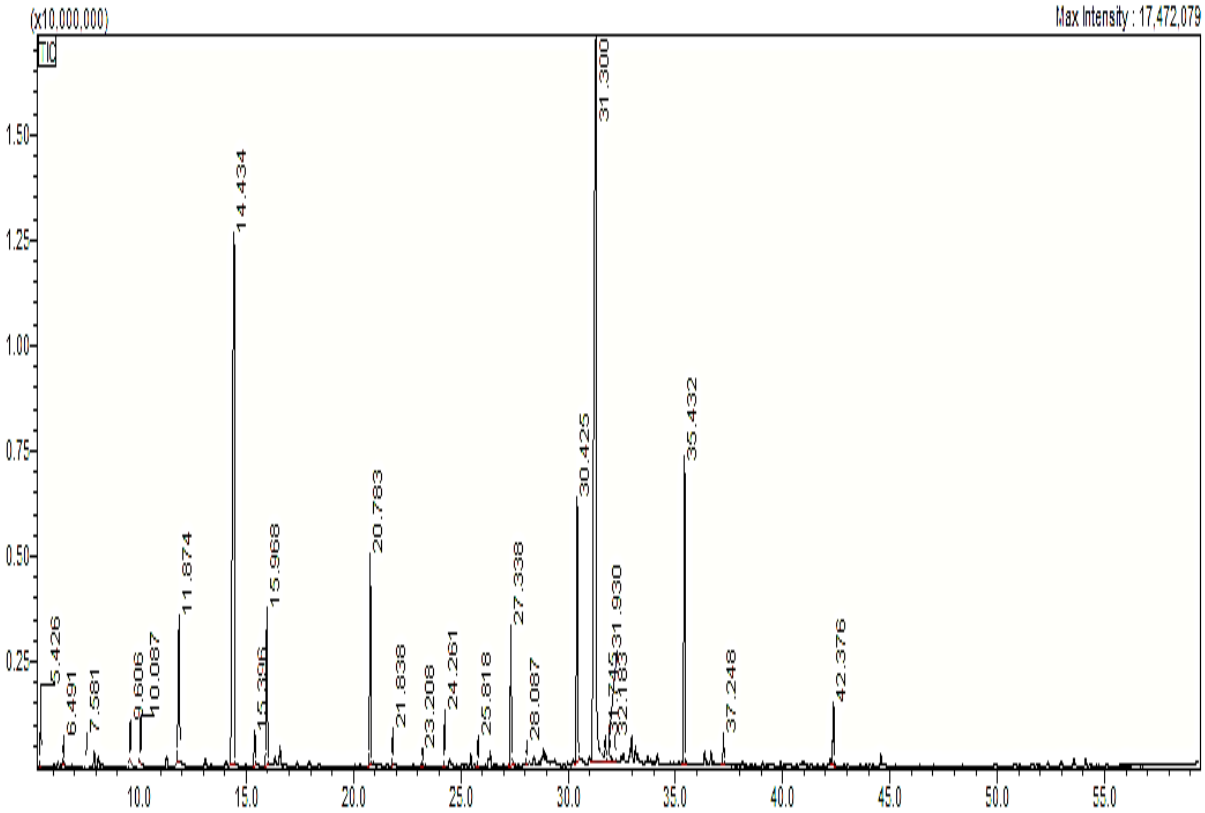


Figure 1. Typical GC-MS chromatogram of *Artemisia judaica* essential oil

Table1. Percent yield and chemical composition of hydro-distilled *Artemisia judaica* essential oil

S. No	Essential oil constituent	[a] RT	[b] RI	Composition (%)
1	Camphene	5.42	943	1.39
2	β-Myrecence	6.49	958	0.47
3	4-Carene	7.58	919	0.55
4	γ-Terpinene	9.60	998	0.97
5	*trans*-Sabinene hydrate	10.09	1041	1.30
6	Linalol	11.87	1082	4.09
7	Camphor (2-Bornanone)	14.43	1121	21.61
8	Borneol	15.40	1138	0.87
9	Terpinen-4-ol	1596	1137	3.90
10	Bornyl acetate	20.78	1277	4.76
11	Myrcenyl acetate	21.84	1292	0.77
12	Hydrocinnamic acid ethyl ester/Ethyl dihydrocinnamate	23.20	1359	0.37
13	Cinnamic acid ethyl ester/*Cis*-Ethyl cinnamate	24.26	1367	1.21
14	Caryophyllene	25.81	1494	0.66
15	*trans*-Ethyl cinnamate	27.33	1367	3.25
16	*trans*-Nerolidol	30.42	1564	6.44
17	(+)-Davanone	31.30	1627	34.32
18	Isoaromadendrene epoxide	31.74	1281	0.97
19	Chamazulene	35.40	1437	7.70
20	3-Ethyl-3-hydroxy-5α-androstan-17-one	37.24	2251	0.68
21	Geranyl-*p*-cymene	42.37	2006	1.64
	Total constituents identified			98.00
	Oil yield (g/100g)			1.08 ± 0.10

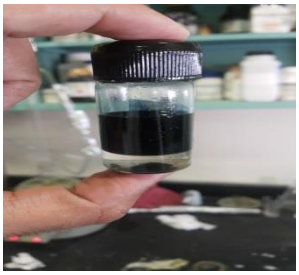
Identification of compounds is based on matching of RI and MS data following Adams (2001) and NIST Library

[a] Chemical compounds are listed in the order of their elution from Rtx-5 MS column.

[b] Retention indices relative to C9–C24 n-alkanes on Rtx-5 MS column.

RT= Retention time

RI = Retention index



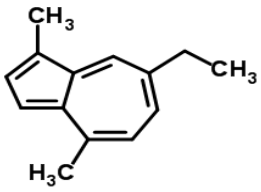


Figure 2.The blue color imparting compound, chamazulene

The quality of the essential oil and its compositional might be influenced by the factors such as geographical location, harvesting time and/or genetic diversities of different *Artemisia* species [2]. In a study of essential oils of *Artemisia judaica* from Algeria, chemical composition was considerably varied, having piperitone as main component with contribution of 61.9% [5] and 53.5% [24]. In an another report, *Artemisia judaica* essential oil from Israel, piperitone, with concentration 4.9-38.5%, was detected as main component followed by camphor (2.6-20.0%) and davanone (0.2-0.9%) in trace amount [25]. There has been found notable variations in principal component of the essential oils isolated of a typical herb harvested from different geographical conditions. *Artemisia judaica* essential oils of Arabian Peninsula are such examples where one can find different chemotypes. Egyptian

Artemisia judaica populations showed different composition for oil yield and main components. In another study, Egyptian *A. judaica* essential oil showed thujone (57.5%) and santolina alcohol (31.4%) as main components [26], whereas in other sample of the same oil, piperitone with concentration as high as 49.1% was found to be the main component [27]. Meanwhile, despite of similar ecological conditions, Jordanian variety of *A. judaica* essential oil exhibited 30.4% piperitone and 16.% camphor [10]. Meanwhile, in *Artemisia* varieties of northern Saudi Arabia spathulenol was dominated as main component with amounts of 30.42% and 28.41% for *A.sieberi* and *A. judaica* varieties, respectively [28]. In fact, such variations in the yield and composition of essential oils can be related to the varied agro-ecological and harvesting conditions, and genetic diversity of plants as well as techniques of oil extraction [29,30].

FTIR analysis of A. judaica essential oil (AEO)

FTIR analysis of AEO was established to identify the type of functional groups/ compounds. With the help of FTIR data (Figure 3, Table 2) we noted most characteristics FTIR bands associated with oil. Strong peaks at 1715 and 1741 cm^{-1} were representative of ketonic functional group for the major compounds davanone and camphor constituting almost 56% of total compounds identified, as confirmed by GC-MS. Similar types of FT-IR peaks for essential oil of some species of *Artemisia* were reported earlier [31].

Table 2: characteristic FT IR bands of *A. judaica* essential oil

Type of vibration	**Functional group present**	**Frequency (cm^{-1})**
C-H Stretching	Aliphatic CH_2	2962.40
Region of double bond stretching	-C=C stretching vibrations of cis olefins	1741.82
	-C=O of Ketone group	1715.40
	-C=O of Amide group	1674.42
Deformation and bending region	-NH bending	1588.73
		1528.25
		1490.86
	-C-H bending vibration of the $–CH_2$	1447.29
	Stretching vibration of –C-O ester functionality	1370.66
Finger Print Region (cm^{-1})	-(CH_2)-,-CH=CH-Overlapping of CH_2 rocking vibration and the out of Plane vibration of cis-di substituted oleifins	1279.16 1247.98 1167.11 952.01 850.41

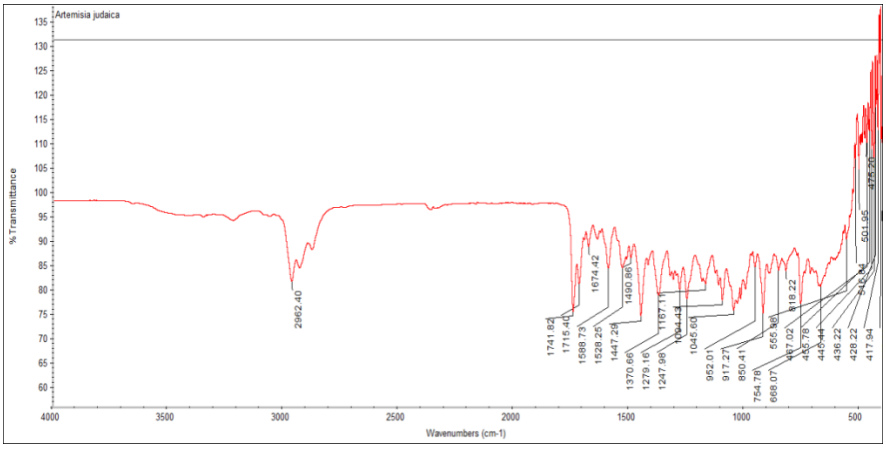


Figure 3. Characteristic FT-IR peaks of *A. judaica* essential oil

Total phenolic compounds (TPC) and DPPHradical scavenging

The concentration of total phenolics in *Artemisia judaica* essential oil was found to be 272.91± 68 mg GAE/100g). Some *Artemisia* species analyzed earlier exhibited similar kind of TPC results, such as *A. absinthium*, *A. arborescens*, *A. campestris*, *A. santonicum*, *A. scoparia* and *A. vulgaris* produced cumulative TPC 161.8 ± 1.41, 100.8 ± 1.41, 201.4 ± 1.41, 293.8 ± 0.02, 332.13 ± 15.8 and 217.46 ± 2.30, respectively [32]. DPPH• radical scavenging power of the tested AEO was observed to be followed in a concentration dependent manner over the conc. range of 0.25 to 10.0 µg/mL (R^2 = 0.997) (Table 3, Figure 4).

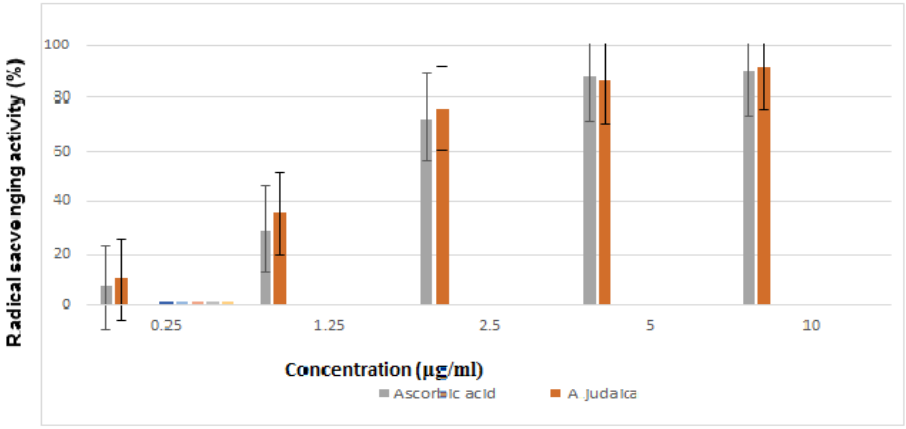


Figure 4. Comparative DPPH scavenging activity of *A. judaica* essential oil

Ascorbic acid was used as standard reagent for comparison. Quite comparable results were found for the *A. judaica* essential oil as compared to standard at similar concentration.

Table 3. % DPPH scavenging assay of *Artemisia judaica* essential oil against ascorbic acid

Concentration (µg/mL)	% DPPH Scavenging Activity of Ascorbic acid (Mean±SD)	% DPPH Scavenging Activity of AEO (Mean+SD)
0.25	7.38±2.46	10.34±1.96
1.25	29.82±1.72	35.85±3.20
2.5	72.55±1.34	76.57+0.73
5	88.08±1.35	86.69±1.67
10	90.49±0.74	92.03±1.12

Cumulative percentage DDPH scavenging of AEO was obtained lowest (10.344±1.969) at 0.25 (µg/mL), and as high as 92.032±1.120 considering 10(µg/mL) concentration. Ability of AEOto reduce the DPPH might be attributed to the presence of principal component davanone (34.32%) and camphor (21.61%). Likewise, antioxidant potential is exhibited to major compounds present in essential oils of different species of *Artemisia* [12].

Kinetics study of Artemisia essential oil (AEO)

Kinetics study of AEO)was carried out to evaluate the performance of extraction technique by utilizing % DPPH results against different time of frame at maximum concentration of 10 µg/mL as shown below-

Table 4. Kinetic study of *Artemisia judaica* essential oil in correlation to antioxidant activity (DPPH %)

Time (min.)	Temperature 25 ^{0}C		
	% DPPH Scavenging Activity (y)	ln(dy/dt)	lny
0	79.35	0	4.35
10	81.68	-1.45	4.40
20	84.57	-1.34	4.43
30	87.85	-1.26	4.47
40	92.01	-1.15	4.52

Kinetic study of AEO was evaluated by utilizing % DPPH data at 0, 10, 20 and 40 min by following formula

$dY/dt = kY^n$

Where Y is antioxidant activity in terms of % DPPH; t is the reaction time; k is reaction (extraction) constant and n is the reaction order. The kinetic study of AEO showed that antioxidant activity was affected by time and concentration following second order kinetics (Figure 5).

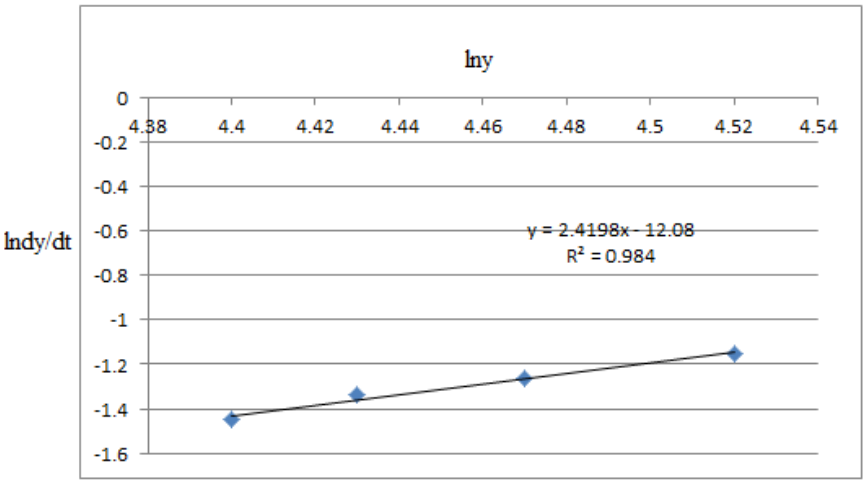


Figure5. Kinetic study of *A. judaica* essential oil at 25^{0}C

Physicochemical properties of AEO

Blue colored and pleasant odored essential oil of *A. judaica* was evaluated for physicochemical properties and the results are summarized in Table 5.

Table 5. Physicochemical characteristics of *A. judaica* essential oil

Parameters	Contribution
Specific gravity (mg/mL)	0.77
Refractive index	1.4360
Acid value (mg KOH/g of oil)	24.00
Peroxide value (mequiv/kg of oil)	43.30
Saponification value (mg of KOH/g of oil)	186.60

Specific gravity and refractive index of presently analyzed oil was found to 0.77 and 1.436, respectively which are close to *Artemisia* essential oils analyzed earlier at room temperature [33].

Acid value is the amount of acid present in an oil [34]. Acid value significantly determines the free fatty acid present in oil and its edibility i.e lower the free fatty acid present in oil better the quality [35]. Acid value of AEO was determined to be 24, which was found to be similar to *A. gmelinii* species [33]. The values obtained in our study was more than the permissible limit (10 mg KOH/g of oil) to be used for dietary purpose [36].

Peroxide value reflects the rancidity of oil upon oxidation under external atmosphere. The high peroxide value (43.3 meq/kg) indicated in our study might be due to extraction of oil at accelerated conditions [37].

Essential oil saponification values are in accordance with some literature suggested [38]. In our present research saponification value of AEO oil was 186.66 (mg of KOH/g of oil). Saponification values of almost all plants essential oils lies between 188-196 (mg of KOH/g of oil) and not useful in soap industries as these values are less than required (>300) [36].

CONCLUSION:

The essential oil yield from aerial parts of *A. judaica* was reasonably good. The tested oil revealed the identification of 21 chemical components, representing 98% of the total oil composition with (+)-davanone (1) as the principal component (34.32%) followed by camphor (21.61%). Through kinetic modeling, a fairly good correlation was established between the extraction process of *A. judaica* essential and the antioxidant activity that was actually affected in a time and concentration dependent manner following second order kinetics. Further, acceptable physicochemical properties such as specific gravity, refractive index, acid value, peroxide value and saponification value of the oil were noted. The findings of this study revealed that

endemic species of *Artemisia* has significant potential for nutra-pharmacetical applications.

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