doi.org/10.36721/PJPS.2025.38.2.REG.13233.1

HPLC-DAD phytochemical revelation, antimicrobial simulation and *in-vitro* bioactivity of endemic herb extracts

Sarah Alharthi[1,2]*, Rokayya Sami[3], Mohamed Sabri Bensaad[4], Necer Abdeldjabar[4], Mohamed Amine Kahoul[5], Roqayah Kadi[6], Ruqaiah Bedaiwi[7], Abeer ALmasoudi[8], Manal Almalki[9] and Abdullah Izmirly[10,11]

[1]Department of Chemistry, College of Science, Taif University, Taif, Saudi Arabia
[2]Research Center of Basic Sciences, Engineering and High Altitude, Taif University, Taif, Saudi Arabia
[3]Department of Food Science and Nutrition, College of Sciences, Taif University, Taif, Saudi Arabia
[4]Faculty of Natural and Life Sciences, University Batna, Fesdis, Batna, Algeria
[5]Laboratory of Food Sciences (LSA), Institute of Veterinary Sciences and Agronomic Sciences, University Batna, Batna, Algeria
[6]Department of Biological Sciences, College of Science, University of Jeddah, Jeddah, Saudi Arabia
[7]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk, Saudi Arabia
[8]Food Science Department, College of Science, Branch of the College at Turbah, Taif University, Taif, Saudi Arabia
[9]Chemistry Department, College of Science, Taibah University, Al-Madinah Al Munawarah, Saudi Arabia
[10]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[11]Special Infectious Agents Unit BSL3, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract: Phenolic compounds form the largest group of phytochemical compounds in plants. They comprise nearly 8.000 molecules divided into around ten chemical classes, and more than ten classes have already been shown to have pharmacological potential. In this context, this study tested the *in-vitro* bioactivity, antimicrobial aspect and vegetal compounds of *Artemisia herba-alba* using HPLC-DAD test. Data indicated that the hemostatic effect of the chloroform fraction was more pronounced and reached a reducing rate of clotting time of 70.91% for the highest tested dose. The photoprotective effect of this fraction was considered high when compared to petroleum ether. The initial phytoscreening revealed the presence of several classes of secondary metabolites. The quantification showed a high amount of flavonoids (88.05±1.94 µg quercetin/mg fraction) in chloroform fraction, while petroleum ether fraction contained more tannins (39.55±1.46 µg tannic acid /mg fraction). HPLC DAD screening revealed the presence of several flavonoids and phenolic acids compounds while the antimicrobial simulation showed a non-negligible effect of 3 tested compounds on several microbial strains. This work revealed the phytochemical aspect of certain extracts of *A. herba-alba* but also its procoagulant, photoprotective and antimicrobial properties.

Keywords: Antimicrobial simulation; hemostatic; phytoscreening; photoprotective; secondary metabolites

Submitted on 13-08-2024 – Revised on 16-01-2025 – Accepted on 29-01-2025

INTRODUCTION

Medicinal plants are a category of herbs used for their particular curative properties to human and even animal health, that's why it is considered in some countries as a form of ancestral and familial therapy intended for the treatment of benign pathologies (Ahn *et al.,* 2024). Nowadays, herbal medicines, like all other medicines, are dispensed by community pharmacies and 50% of the small molecules marketed for the treatment of cancer and the most effective drugs for treating influenza or malaria, are still extracted or derived from plants (Falchetto *et al.,* 2024).

Cancer is the leading cause of death in men and the second leading cause of death in women. Among these categories of cancers, skin cancer known as melanoma manifests itself in the form of skin lesions, with a clear prevalence of 132.000 new cases / year and are often due to intense and prolonged exposure to the sun (Siegel *et al.,* 2024). Surgery is the main treatment for most skin cancers, especially for patients with basal cell or squamous cell carcinomas, but this practice remains risky with many side effects (Silverstein *et al.,* 2024). Let's not forget that despite the benign aspect of certain pathologies such as Meningiomas; that develop in the meninges of central nervous system, Hemangiomas; that grow from blood vessels and osteoma; a bone tumor, often located in the paranasal sinuses, the risk of becoming malignant still remains high due to growth factors, mutation, exposure to radiation and other genetic and environmental conditions (Hernandez *et al.,* 2022; Suárez-Fernández & García-Pola, 2024). In this context, phytotherapy has proven its capacity to prevent cancer and among the most used plants for this purpose are turmeric, green tea and milk thistle. These plants have in common that they have been the subject of numerous preclinical studies that have highlighted their ability to interfere at several stages of the cancerization processes (Falchetto *et al.,* 2024). The best example to illustrate it is Hello Sunshine, which is a body and face sunscreen oil

**Corresponding author:* e-mail: rokayya.d@tu.edu.com

with a 100% natural and plant-based formula. In this context, the photoprotective assay allows scientists to check if a candidate plant will be able to stimulate the photoprotective mechanism, namely; avoidance, photorespiration, dissipation and anti-oxidation in order to integrate their phytocompounds in a safe way during formulation process of bio-sunscreen to prevent sunburn, aging and skin cancer (Timm *et al.,* 2024).

It is also well known that plants have an innate immunity that allows them to perceive aggressive microorganisms, and numerous studies attest to the antibacterial properties of certain plants or natural substances. Garlic, tea tree, propolis or grapefruit seed extract are among the best "natural antibiotics", capable of effectively fighting certain infectious diseases like skin abscesses, infectious diarrhea, vaginitis and stomach infection (Refaey *et al.,* 2024). That's why scientists agree to say that the bioactive compounds in plants could be effective to be formulated as antimicrobial drugs and let's not forget that bioinformatics approaches are representing these days, a crucial initial step to characterize the molecular process of this antimicrobial effect (Sakagianni *et al.,* 2023).

Hemostasis includes all the natural phenomena that allow the bleeding to stop in the event of injury, vascular lesion, shock or surgery. However, in certain pathological cases, like hemophilia and Von Willebrand disease, a disruption can occur, leading to an upheaval in the coagulation process. However, plants can act as a procoagulant like *Eclipta alba* and *Rosa chinensis* or anticoagulant agent like *Thymus vulgaris* and *Cinnamomum cassia*, thus regulating this process, especially in the case of pregnant women who may have certain coagulation disorders and lead to miscarriages (Bensaad *et al.,* 2021). In this context, the hemostatic assay allows scientists to check if a candidate plant will accelerate or delay the implication of platelets and fibrinogen that will come into action during coagulation process in order to "plug" the breach by forming the platelet plug in blood vessels (Katz *et al.,* 2024).

The genus *Artemisia* is one of the most important and widely distributed genera of the Asteraceae family, comprising 522 small herbs and shrubs (Hussain *et al.,* 2024). The pharmaceutical companies have exploited many compounds extracted from different types of this genus like; *Artemisia Annua* or *Artemisia verlotiorum* (Bisht *et al.,* 2021). Indeed, the species of this genus can resist drying and have a non-negligible tonic and stimulating effect on the digestive system, specifically on the secretion of gastric juice, but also an antioxidant, hepatoprotective and antiviral effects (Bisht *et al.,* 2021; Hussain *et al.,* 2024).

Moreover, these two *in-vitro* methods were chosen, since species of the genus *Artemisia* are well known for their hemostatic effect but we wanted to check if the same effect will be obtained from the same plant but belonging to another geographical context and specific harvesting time. On the other hand, any experimental assay has been used yet to evaluate the photoprotective capacity of *Artemisia* species and in particular of *A. herba-alba*.

In this article, we sought to highlight the potential of *Artemisia herba alba* extracts in terms of activities and to characterize their qualitative, yield and chemical constituents from the aerial part (leaves and flowers).

MATERIALS AND METHODS

Plant origin and fractionation

All chemicals, standards used in this work were as follow: acetate ethyl, acetonitrile (C_2H_3N), caffeic acid (C_9H8O4), calcium chloride ($Cacl_2$), catechin ($C_{15}H_{14}O_6$), cinnamic acid ($C_9H_8O_2$), chloroform, distilled water, gallic acid ($C_7H_6O_5$), methanol, petroleum ether, sulfuric acid (H_2SO_4), tannic acid ($C_{76}H_{52}O_{46}$), trans-ferulic acid ($C_{10}H_{10}O_4$), trans-cinnamic acid ($C_9H_8O_2$), n-butanol, vanillin ($C_8H_8O_3$), 4-hydroxybenzoic acid ($C_7H_6O_3$).

Plant origin and fractionation

For the proper achievement of this work, The samples of *A. herba-alba* were collected in the municipality of Oued Taga, Batna region, Algeria (GPS coordinates: latitude 35.255727; longitude 6.245181) and was identified by experts under a voucher specimen (AHA/2023/MSBMA). The extraction process was conducted using methanol (95%) as main solvent and 400g of the plant. Maceration was done 3 times with MeOH-H2O (ratio 70:30) for 72h. Then, 4 fractions were obtained using solvents from non-polar to polar respectively; petroleum ether (0.72%), chloroform (0.89%), acetate ethyl (1.58%) and n-butanolic (2.45%). Fig. 1 provides a clear representation of this species and evaluations.

Qualitative and quantitative yield determination

The initial screening for the presence or absence of 10 secondary metabolites classes in the main methanolic extract was performed following standard protocols (Li *et al.,* 2024). However, the estimation of total phenolic, flavonoids and total tannins proportion were established as previously reported by Farag *et al.* (2020), in which these chemicals were used as controls; gallic acid, tannic acid and quercetin.

HPLC-DAD screening

The HPLC analysis of *A. herba-alba* extracts was performed by a Shimadzu HPLC system (manufacturer: Shimadzu Seisakusho Co., Ltd. Kyoto, Japan (model 2010), column: univerSil HS C18 (Forties Technologies, UK), dimensions: 150 mm x 4.6 mm x 5µm particle size), using a volume of injection of 20µl of each extract. Briefly, The elution flow rate was 0.5 ml/min (wavelength at 280 nm, gradient program: 10-17% for 0-5min; 19-22% for 5-15min and 26-30% for 15-36 min), while mobile phase

consisted of two solvents; acetonitrile and H2SO4 in water (0.2N). The UV detection was set at a wavelength equal B). The UV detection wavelengths were performed at 280 nm and 9 standards were used for this purpose (Bensaad *et al.,* 2021).

In vitro biological quality

Haemostatic test

This test was performed on blood plasma from a healthy person with the petroleum ether and chloroform fractions as an evaluation sample, using the following concentrations (50, 100, 200, 400 and 800µg/ml). The principle of this test is to measure the clotting time (CT) of decalcified plasma after recalcification (Bensaad *et al.,* 2021). Indeed, blood was collected on sodium citrate tubes from healthy subjects, then plasma from blood centrifuged at 3000 rpm for 15 minutes. The tubes are kept in a water bath at 37°C. We add 200µl of plasma and 200µl of calcium chloride (Cacl2) at 0.025M to each tube. Noting that the stopwatch was started as soon as the plasma penetrated into the tube.

Furthermore, this formula was used to generate the reducing % of CT:

$$S\% = [\frac{(T1 - T2)}{T1}] \times 100$$

In which,

S: reducing % of CT;

T1: CT of control;

T2: CT of tested samples.

Photoprotective test

A spectrophotometric method was used to determine sun protection factor (SPF) of petroleum ether and chloroform extracts of *A. herba-alba*, dissolved in methanol at the dose (4 mg/ml). Then, spectral transmittance was measured by spectrophotometer at UV wavelengths 290–320 nm with 5 nm of intervals (Bensaad *et al.,* 2021).

Furthermore, this formula was used to generate SPF values:

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

In which:

CF: correction factor (= 10);

EE: erythemal action spectrum;

I: solar simulator illuminance intensity;

EE x I: constant values conforming to Sayre *et al.* (1979).

Antimicrobial capacity simulation

For this test, AVC pred, anti Bac-Pred and MICF platforms were employed respectively to simulate antiviral, antibacterial and antifungal capacities of our identified chemicals. Noting that details about the database and functional aspect of these platforms are described in our previous work (Sakagianni *et al.,* 2023). Noting that these 3 platforms use a quantitative structure–activity relationship (QSAR)-based models and internal database with programmed parameters and a precise algorithmic system to generate the probability of activity (PA) of each tested compound but also their probability of inactivity (PI).

Ethical approval

Our work was conducted according to the Algerian National Blood Agency respecting the Ordinance No. 68-133 of May 13, 1968 on the general organization of blood transfusion and transfusion establishments.

STATISTICAL ANALYSIS

All experimental measurements were performed in triplicate and are expressed as the mean ± standard deviation of three analyses (mean (SE) ± standard deviation). Statistical analysis was performed using one-way analysis of variance (ANOVA) and student's t-test. If the overall P-value was found significant at ($P<0.05$). All statistical analysis and significance of correlation between variables were performed using the software GraphPad Prism version 8 (California, USA).

RESULTS

Qualitative and quantitative yield determination

The initial phytoscreening of the methanolic crude fraction revealed the presence of several classes of secondary metabolites like flavonoids, tannins, triterpenes saponins and alkaloids (table 1). However, in our work, steroids, mucilages and cardiotonic glycosides were absent.

In term of quantification, data showed a high amount of total polyphenols proportion (TPP) in chloroform fraction (135.82±1.7цg GAE/mg), in which flavonoids represented (88.05±1.94 цg quercetin/mg fraction) and was statically considered significant ($p<0.01$) However, petroleum ether fraction contained more tannins (39.55± 1.46 цg tannic acid /mg fraction) (table 2).

HPLC-DAD screening

As shown in fig. 2 and 3, the HPLC analysis revealed the presence of several flavonoids and phenolic acids compounds and this proportion was more important and significant ($p<0.01$) in chloroform fraction, in which vanillin (4.78±0.81 (µg/mg) was predominant, then by caffeic acid (1.89±0.11) and catechin (1.77±0.04µg/mg). On the other hand, only 7 compounds have been identified in petroleum ether fraction, in which trans-cinnamic acid was predominant (6.95±1.48µg/mg), then 4-hydroxybenzoic acid (1.99±0.62µg/mg) and finally vanillin (1.95±0.26µg/mg) (table 3).

Haemostatic test

In regards to chloroform fraction, the hemostatic effect evolved as pro-coagulant and was set up in dose dependent manner to reach an accelerated coagulation process for a clotting time of 4mg/ml, which refer to a reducing rate of 70.91%. However, results were modest for petroleum ether

fraction and the maximum reducing rate of hemorrhage was 45.39% for the maximum tested doses as indicated in table 4.

Photoprotective test

For this test, only chloroform fraction was able to generate an optimal SPF score of 39.717 (table 5), in a significant way ($p<0.01$), which correspond to a high photoprotective effect, However, a negligible SPF factor was found using petroleum ether factor, which suggest that this fraction could not be suitable to integrate the actual formulation process of dermatologic sunscreen.

Antiviral, antibacterial and antifungal properties prediction

For this simulation, 3 chemicals were used as reported in Table 6. Indeed, compounds 2 and 3 were the only ones acting on viral strains and their anti-HIV and anti-HCV effects were modest and almost the same but the anti-HHV of compound 2 was more pronounced (PA= 0.414) as reported in table 7.

In regard to bacterial strains, a remarkable antibacterial effect was expressed by compound 1 (PA=0.703) against *Pseudomonas fluorescens* while the effect of the other chemical was negligible against this strain. Noting that a modest antibacterial effect was exhibited by compound 2 and 3 against *Streptococcus viridans* with a respective value of PA= 0.498 and PA= 0.427.

In regard to fungal strains, the same constant was observed and compound 1 exerted a significant effect (PA=0.579) on *Candida dubliniensis*. Noting that compounds 2 and 3 were more active on *Epidermophyton floccosum,* when compared to the 1st compound with a respective value (PA=0.320; PA=0.353).

DISCUSSION

***Qualitative and quantitative* yield determination**

The absence of steroids, mucilages and cardiotonic glycosides in our herb could be linked to the geographical and climatic context of the region (Hussain *et al.,* 2024). The genus *Artemisia* has been reported to be rich in secondary metabolites such as flavonoids, caffeoylquinic acids, coumarins, essential oils, sterols and acetylenes (Bisht *et al*., 2021; Hussain *et al.,* 2024). As well, the aerial part of *A. Campestris* L is rich in phenolic acids, alkaloids and tannins (Ivănescu *et al.,* 2021). In the same context, it was also reported that *A. Annua* contains a high amount of volatile oils, terpenes and flavones coumarins (Bisht *et al*., 2021; Ivănescu *et al.,* 2021; Hussain *et al.,* 2024).

The quantified proportion of polyphenols in our herb is also interesting since another species of the same genus called *A. campestris* showed an interesting value of TPP ranged from 74.75±0.01 to 88.61±0.22mg EAG/g of methanolic fraction; while tannins represented 35.29-70.59mg EC/g of methanolic fraction (Trifan *et al.,* 2022). In is also interesting to note that a formulation process using *A. Annua* revealed a high flavonoid proportion in powder and capsules, in which this class represented 1.6±0.1 mg quercetin equivalent (Ferreira *et al.,* 2010).

Noting that more than 40 flavonoids have been isolated from *A. annua*, including luteolin, quercetin, or casticin and this information is important since flavonoids alone have weak antimalarial effects *in vitro*, but in the presence of other phenolic compounds, especially phenolic acids, this effect can increase 50% (Ferreira *et al.,* 2014). Another work showed that *A. Herba alba* essential oil analyzed by GC/MS has β-thujone (23.92%) and chrysanthenone (17.4%) (Dob & Benabdelkader, 2006).

Another approach using HPLC-DAD showed that 3 species of the same genus respectively *A. judaica*, *A. monosperma*, and *A. sieberi* have common phenolic compounds with our species, such as quercetin, gallic acid, and tannic acid (Salih *et al.,* 2023). Another study, this time made by a Moroccan team showed that *A. mesatlantica* essential oil contained β-thujone (56.33%) is the main compound identified followed by camphene (7.48%) and camphor (4.17%) (Polito *et al.,* 2024).

It is interesting to note that *Artemisia* species have several therapeutic applications, especially in Asian countries. Indeed, these herbs are used as a hemagogue, antispasmodic and haemostatic agents. They are also prescribed against menstrual disorders, leucorrhea, haemorrhagic dysentery, hemoptysis, epistaxis, metrorrhagia, vomiting, colic, neuralgia and impetigo (Bisht *et al*., 2021; Hussain *et al.,* 2024). *Artemisia* species such *A. absinthium* possess also astringent properties and are mainly used externally, especially to treat wounds, sores or hemorrhoids due to the presence of high flavonoids and saponins proportion (Hashempur *et al.,* 2017). However, the same research revealed that the chloroform fraction of *A. absinthium* can reduce prothrombin time (PT) more effectively than our herb by reducing clot lysis activity of strepotokinase, which suggest that *A. absinthium* may contains more bioactive / potent phyto-compounds (Bisht *et al*., 2021). Noting that tannins of *Artemisia* can also be used internally, for example for the treatment of hemorrhagic diarrhea, Gastrointestinal bleeding and this metabolite can also accelerate the activity of coagulation factor X (Açıkgöz & Açıkgöz, 2013). Noting that the principal constituent of *Artemisia* species named *Artemisinin* and its derivatives can act very quickly on intra-erythrocyte malaria parasites, thus allowing the elimination of this parasite (Tilley *et al.,* 2016). This information is interesting, since our herb exhibited better pro-coagulant effect when compared to the methanol fraction *Artemisinin,* which maximum shortening rate of clotting time was only (27.37%) (Wang *et al*., 2011).

Table 1: Results of the preliminary analysis of methanol extract of *A. herba-alba*.

Phytochemicals classes	Respective experiment	Analysis Results
Tannins	Ferric chloride approach	+
Steroids	Liebermann Burchard approach	-
Flavonoids	Shinoda's approach	+
Anthocyanins	Bornträger approach	+
Triterpenes	Liebermann Burchard approach	+
Alkaloids	Dragendroff approach	+
Quinones	NaOH approach	+
Saponins	Foam approach	+
Mucilages	Ethanol approach	-
Cardiotonic glycosides	Keller Kiliani approach	-

(-) = absent; (+) = present

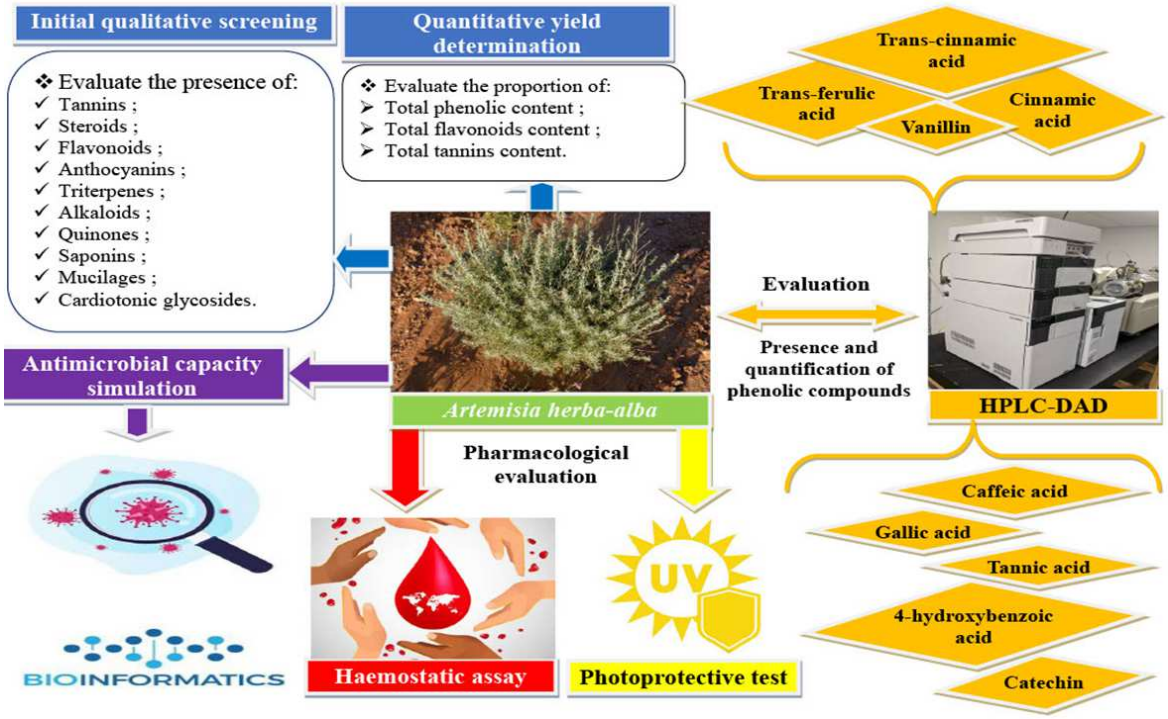


Fig. 1: Illustrative photo representing *A. herba-alba* with evaluations.

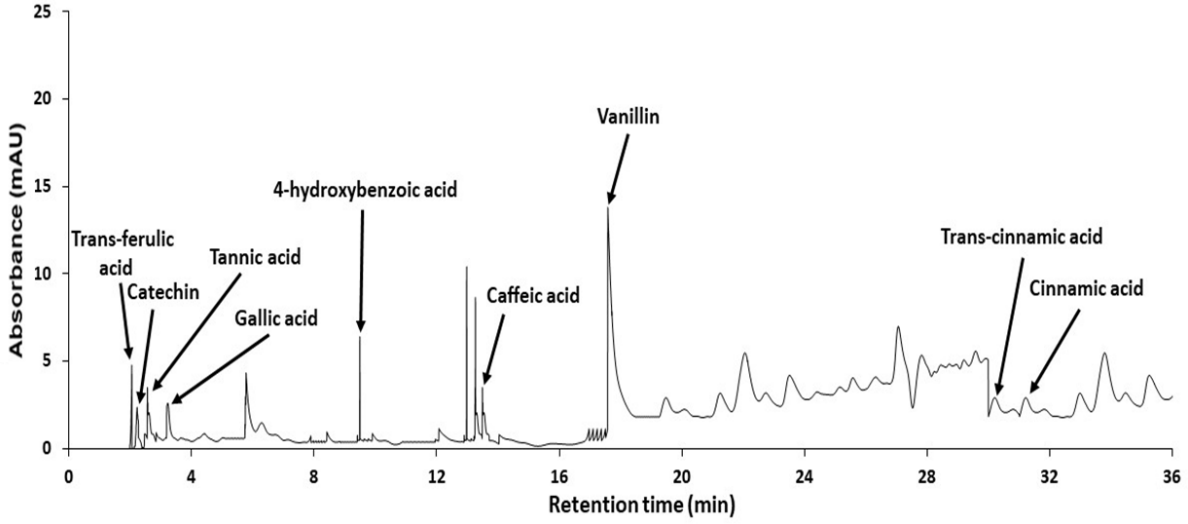


Fig. 2: Chromatograms of chloroform fraction of *A. herba-alba* at 280 nm.

Table 2: Quantitative analysis result of petroleum ether and chloroform extracts of *A. herba-alba*.

Category of extract	Total polyphenols proportion µg GAE/mg fraction	Flavonoids proportion (µg quercetin/mg fraction)	Total tannins proportion (µg tannic acid /mg fraction)
Petroleum ether	112.04 ± 2.66[b]	71.19 ± 1.52[b]	39.55 ± 1.46[a]
Chloroform	135.82 ± 1.7	88.05 ± 1.94	30.74 ± 1.28

Data ± SD (n=3), followed by student's t-test. Significance at [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ vs chloroform extract.

Table 3: HPLC-DAD quantitative results of the detected compounds in *A. herba-alba* fractions.

Tested chemical	Proportion (µg/mg in chloroform fraction)	Proportion (µg/mg in petroleum ether fraction)
Gallic acid	1.48 ± 0.15[c]	0.27 ± 0.02
Catechin	1.77 ± 0.04	-
Vanillin	4.78 ± 0.81[c]	1.95 ± 0.26
Trans-ferulic acid	0.22 ± 0.05	-
Cinnamic acid	-	0.83 ± 0.09
Trans-cinnamic acid	1.07 ± 0.13[c]	6.95 ± 1.48
4-hydroxybenzoic acid	0.74 ± 0.01[b]	1.99 ± 0.62
Tannic acid	1.38 ± 0.17[ns]	1.74 ± 0.38
Caffeic acid	1.89 ± 0.11[b]	0.58 ± 0.06

Data ± SD (n=3), followed by student's t-test. Significance at [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ vs petroleum ether extract. [ns]: no significance.

Table 4: Effect of petroleum ether and chloroform fractions of *A. herba-alba* on clotting time.

Treatment	Doses (mg/mL)	Mean clotting time (sec)	Reducing rate of clotting time (%)
Control	/	158.14 ± 10.87	/
Chloroform fraction	0.125	99.25 ± 3.77[b]	37.23%
	0.25	87.42 ± 3.91[c]	44.71%
	0.5	80.04 ± 3.17[c]	49.38%
	1	68.32 ± 3.11[c]	56.79%
	2	55.76 ± 2.48[c]	64.74%
	4	46 ± 2.95[c]	70.91%
Petroleum ether fraction	0.125	175.83 ± 7.04[ns]	0%
	0.25	146.33 ± 4.26[ns]	7.46%
	0.5	122 ± 4.59[a]	22.85%
	1	101 ± 4.82[b]	36.13%
	2	95.73 ± 4.05[b]	39.46%
	4	86.36 ± 3.28[c]	45.39%

Data ± SD, n=3. One way ANOVA with multiple Dunnet's test. Significance at [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ vs control. [ns]: no significance.

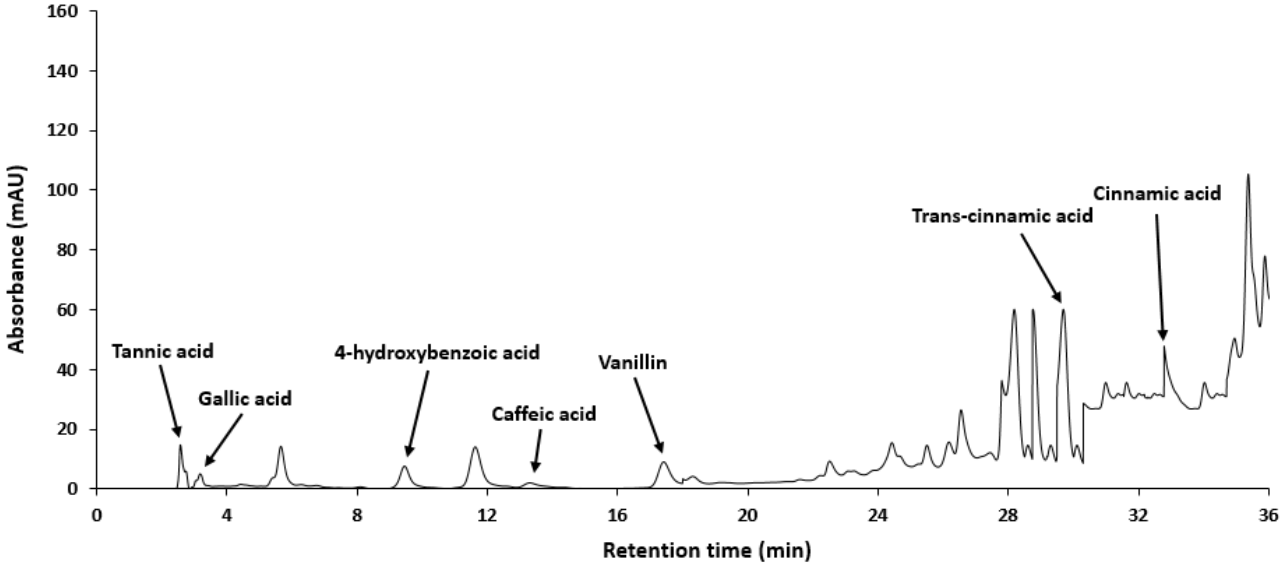


Fig. 3: Chromatograms of petroleum ether fraction of *A. herba-alba* at 280nm.

Table 5: Photoprotection results (SPF) from *A. herba-alba* fractions.

Fixed parameters		*A. herba-alba* fractions	
Wave length λ (nm)	EE (λ)x I(λ) (normalized)	Chloroform fraction (SPF)	Petroleum ether (SPF)
290	0.0150	0.559^{C}	0.028
295	0.0817	3.127^{C}	0.267
300	0.2874	12.781^{C}	1.095
305	0.3278	15.340^{C}	1.381
310	0.1864	4.992^{C}	0.752
315	0.0837	2.512^{C}	0.329
320	0.0180	0.406^{C}	0.045
Total	/	39.717^{C}	3.387

Data ± SD (n=3), followed by student's t-test. Significance at $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ vs petroleum ether extract.

Table 6: Structural features of the tested chemical.

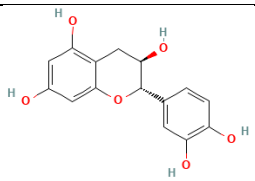
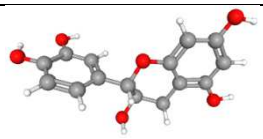
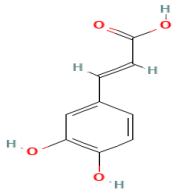
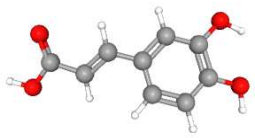
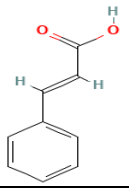
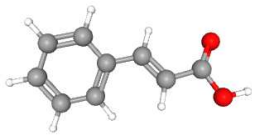
Tested chemical	Molecular Formula	2D structure	3D structure	PubChem ID
Catechin (1)	$C_{15}H_{14}O_6$			73160
Caffeic acid (2)	$C_9H_8O_4$			689043
Trans-cinnamic acid (3)	$C_9H_8O_2$			24893022

Table 7: Antimicrobial properties of the tested compounds.

Chemical	Antiviral capacity	PA	Antibacterial capacity	PA	Antifungal capacity	PA
			Pseudomonas fluorescens	0.703	Candida dubliniensis	0.579
1	NA	/	Listeria monocytogenes	0.445	Trichophyton mentagrophytes	0.215
			Kocuria rhizophila	0.300	Epidermophyton floccosum	0.087
	HIV	0.430	Streptococcus viridans	0.498	Epidermophyton floccosum	0.320
2	HCV	0.511	Pseudomonas fluorescens	0.396	Trichophyton mentagrophytes	0.203
	HHV	0.414	Yersinia pestis	0.386	Mucor	0.127
	HIV	0.453	Yersinia pestis	0.441	Epidermophyton floccosum	0.353
3	HCV	0.477	Streptococcus viridans	0.427	Mucor Candida rugosa	0.270
	HHV	0.265	Pseudomonas fluorescens	0.392		0.132

PA: Probability of activity. HIV: Human Immunodeficiency Virus; HCV: Hepatitis C Virus; HHV: Human Herpes Virus; NA: Not available; NA: No Active.

The high proportion of vanillin, caffeic acid and catechin in chloroform fraction may explain the hemostatic capacity recorded. Indeed, it was reported that a high proportion of vanillin, caffeic acid and catechin in plant fraction can considerably promote in a dose-dependent manner the intrinsic and extrinsic coagulation system, increasing blood vessel permeability by acting on cytosolic and mitochondrial Ca^{2+}, which will in return significantly increase procoagulant platelets and thus the recovery time linked to wounds or during surgery, which was previously proved on highly vascularized testicular tissue of rats (Luo et al., 2017; Yaneva & Ivanova, 2020; Du *et al.,* 2024).

Our chloroform fraction exibited a highly significant SPF score when referring to the European commission recommendation on sun product categories (Renner, 2021). Several scientific teams noted that some plant's extracts and oils rich in flavonoids can act in a synergistic way with other classes such quinones and triterpenes to generate an optimal effect against UV rays, that's why are currently

advertised as being slightly photoprotective (Bensaad *et al.,* 2021).

Noting that, nowadays, Phenol peeling is a treatment that involves applying a group of phenolic acid formulated in a cream to the skin of the face in order to destroy the superficial layers by coagulation of proteins and liquefaction of the first layers of the epidermis and dermis, which in result can considerably limit the emergence of melanoma (Pop & Diaconeasa*,* 2021).

Several plants like turmeric, green tea and milk thistle has an important proportion of saponins and terpenes which were subjected to numerous preclinical studies that have highlighted their ability to interfere at several stages of the dermis cancerization processes but also integrated in current medicinal chemistry as anti-cancer agents (Aydoğmuş-Öztürk *et al.,* 2020). A plant with a high proportion of vanillin and caffeic acid can justify its photoprotective capacity, since vanillin may suppress the downstream step of multiple murine double minute gene 2 (MDM2) in UVB irradiation-induced p53 activation, which is very important since MDM2 is a critical component of the responses to both ionizing and UV radiation (Lee *et al*., 2014; Agilan *et al.,* 2016). Moreover, caffeic acid can defend phospholipidic biomembranes from UV light induced peroxidation (Lopes *et al.,* 2021).

The phenolic acid nature of compound 2 and 3 can actually justify the antiviral results. Indeed, it is well known that phenolic acids can act against both DNA and RNA viruses and several studies revealed that plant extracts which possess a high amount of phenolic acids are able to inhibit the activity of the main protease of the virus 3CL pro and control the activity of the replication complex and thus limit different levels of viral infection, entrance and even translation of viral proteins (Kiokias & Oreopoulou, 2021; Wang *et al.,* 2023; Adeosun & Loots, 2024).

Another study underlined the antiviral effect of *Artemisia* species against HCV using JFH-1 strains (Obeid *et al.,* 2013), while a work made by an African team suggested that *A. annua* tea infusions can effectively inhibit and suppress HIV (Lubbe *et al.,* 2012). In addition, scientists from Germany have launched *in vitro* studies to test the effectiveness of plant extracts against SARS-CoV-2. The team evaluated its activity against SARS-CoV-2 in a monkey lung cell model, and suggests that *Artemisia* herbal teas may have an effective antiviral effect (Le-Trilling *et al.,* 2022).

Let's not forget that *Pseudomonas* are a common cause of nosocomial urinary tract infections, particularly after urological procedures or obstructive uropathy (Huang *et al.,* 2024). In addition, *Pseudomonas* often colonizes the urinary tract in catheterized patients, especially those who have received broad-spectrum antibiotics (Derickx *et al.,* 2024). The flavonoid nature of compound 1 may explain this antibacterial effect since literature showed that this class can inhibit the growth of pathogenic Gram-positive bacteria (Xie *et al.,* 2015).

The pathogenic character of this strain could explain the emergency to elaborate more effective antimicrobial drugs since *Streptococcus viridans* live in the mouths of healthy people, but can invade the bloodstream, especially in people with periodontal inflammation, and infect the heart valves, causing endocarditis (Arjun *et al.,* 2024).

The flavonoid nature of compound 1 may explain its antifungal effect, since flavonoids possess potent anti-Candida effect by inhibiting, growth and proliferation of this pathogenic strain (Nguyen *et al.,* 2021). This information is important since this microscopic fungus, naturally present in the human body, can cause severe chronic infections, especially vulvovaginitis (Kolekar *et al.,* 2019) and is particularly potentially dangerous in fragile or immunocompromised people (Chang *et al.,* 2018), hence the importance of knowing how to protect against it and knowing the symptoms. Noting that compounds 2 and 3 were more active on *Epidermophyton floccosum,* and thus considered slight effect recorded on this pathogenic filamentous fungus strain (Kebede & Shibeshi*,* 2022).

CONCLUSION

In this modest work, chronological phytoscreening approaches were performed to reveal the composition of two fractions of *A.herba-alba* in which flavonoids and phenolic acids were in the majority. In addition, the pharmacological quality of this herb was set up using two *in-vitro* methods and revealed non-negligible effects, especially for chloroform fraction. In addition, the antimicrobial simulation showed some interesting effect of this herb fraction, which could mean that the phenolic constituents of our herb acted in synergistic way to generate these effects. However, future *in-vivo* approaches, especially wound healing capacity test (both excision and incision methods) are mandatory to confirm the hemostatic and procoagulant effect of our herb, while more advanced photoprotective assays will reveal and confirm the effect of our herb on the pigmentation level of skin and thus to formulate more advanced sunscreen with less side effects. This work revealed the full phytochemical aspect of *A. herba-alba* using referenced standards but also the curative aspect of this plant using two fractions, and interesting data were found, especially with the chloroform fraction. However, the antimicrobial simulation revealed some new aspect of the potential of some phenolic compounds on some microbial strains. However, future in-vivo studies must be performed to confirm the data of this preliminary work.

ACKNOWLEDGMENTS

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-10).

FUNDING

This research was funded by Taif University, Saudi Arabia, Project No. (TU-DSPP-2024-10).

REFERENCES

Ahn JY, Chu H, Leem J and Yun JM (2024). Effectiveness and safety of traditional herbal medicine on cardiac arrhythmic condition: A systematic review and meta-analysis of randomized control clinical trial. *Medicine (Baltimore)*, **103**(23): e38441.

Acıkgoz SK and Açıkgoz E (2013). Gastrointestinal bleeding secondary to interaction of Artemisia absinthium with warfarin. *Drug. Metabol. Drug. Interact*, **28**(3): 187-189.

Adeosun WB and Loots DT (2024). Medicinal plants against viral infections: A review of metabolomics evidence for the antiviral properties and potentials in plant sources. *Viruses*, **16**(2): 218.

Agilan B, Rajendra Prasad N, Kanimozhi G, Karthikeyan R, Ganesan M, Mohana S, Velmurugan D and Ananthakrishnan D (2016). Caffeic acid inhibits chronic UVB-induced cellular proliferation through JAK-STAT3 signaling in mouse skin. *Photochem. Photobiol*., **92**(3): 467-474.

Arjun R, Niyas VKM, Hussain F, Surendran S and Mohan V (2024). Clinical and microbiological profile of viridans group streptococcal bacteraemia; experience from South India. *Infez Med*., **32**(1): 37-44.

Aydogmus-Ozturk F, Jahan H, Ozturk M, Gunaydın K and Choudhary MI (2020). Preclinical study of the medicinal plants for the treatment of malignant melanoma. *Mol. Biol. Rep*., **47**(8): 5975-5983.

Bensaad MS, Dassamiour S, Hambaba L, Bensouici C and Haba H (2021). *In vitro* assessment of antioxidant, anti-inflammatory, neuroprotective and antimicrobial activities of Centaurea tougourensis Boiss. & Reut. *J. Pharm. Pharmacogn. Res*., **9**(6): 790-802.

Bisht D, Kumar D, Kumar D, Dua K and Chellappan DK (2021). Phytochemistry and pharmacological activity of the genus Artemisia. *Arch. Pharmacal Res*., **44**(5): 439-474.

Chang EY, Fatima S, Balan S, Bhyravabhotla K and Erickson M (2018). *Candida dubliniensis* abscess: A clinical case and a review of the literature. *Med. Mycol. Case Rep*., **21**: 41-43.

Derickx LAJ, Willemse-Erix D, van Piggelen A, Steegh P, Heijckmann AC, Hermans MHA, de Vocht TF and Wever PC (2024). An outbreak of *Pseudomonas aeruginosa* urinary tract infections following cystoscopy traceable to a malfunctioning drying cabinet. *Infect. Prev. Pract*., **6**(3): 100378.

Dob T and Benabdelkader T (2006). Chemical composition of the essential oil of Artemisia herba-albaAsso grown in Algeria. *J. Essent. Oil Res*., **18**(6): 685-690.

Du C, Fikhman DA, Obeng EE, Can SN, Dong KS, Leavitt ET, Saldanha LV, Hall M, Satalin J, Kollisch-Singule M and Monroe MBB (2024). Vanillic acid-based pro-coagulant hemostatic shape memory polymer foams with antimicrobial properties against drug-resistant bacteria. *Acta Biomater*., **189**: 254-269.

Falchetto L, Bender B, Erhard I, Zeiner KN and Stratmann JA (2024). Concepts of lines of therapy in cancer treatment: Findings from an expert interview-based study. *BMC Res. Notes*., **17**(1): 137.

Farag RS, Abdel-Latif MS, Abd El Baky HH and Tawfeek LS (2020). Phytochemical screening and antioxidant activity of some medicinal plants' crude juices. *Biotechnol. Rep*., **28**: e00536.

Ferreira JF, Luthria DL, Sasaki T and Heyerick A (2010). Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules*, **15**(5): 3135-3170.

Hashempur MH, Khademi F, Rahmanifard M and Zarshenas MM (2017). An evidence-based study on medicinal plants for hemorrhoids in *Medieval persia*. *J. evid.-based complement. altern. med*., **22**(4): 969-981.

Hernandez YB, Gomez KV and Lopez AL (2022). Treatment of benign tumours and related pathologies with radiotherapy: Experience of the general hospital of Mexico. *Rep. Pract. Oncol. Radiother*., **27**(4): 684-690.

Huang X, Ding J, Yang X, Tian B, Yu R, Lyu M, Liu W and Ding Q (2024). Clinical characteristics and prognosis analysis of *Pseudomonas aeruginosa* bloodstream infection in adults: A retrospective study. *Clin Exp Med*., **25**(1): 5.

Hussain M, Thakur RK, Khazir J, Ahmed S and Khan MI (2024). Traditional uses, Phytochemistry, pharmacology and toxicology of the genus *Artemisia L.* (Asteraceae): A High-value Medicinal Plant. *Curr. Top. Med. Chem*., **24**(4): 301-342.

Ivănescu B, Burlec A.F, Crivoi F, Roșu C and Corciovă A (2021). Secondary metabolites from Artemisia genus as biopesticides and innovative nano-based application strategies. *Molecules*, **26**(10): 3061.

Katz D, Farber M, Getrajdman C, Hamburger J, Reale S and Butwick A (2024). The role of viscoelastic hemostatic assays for postpartum hemorrhage management and bedside intrapartum care. *Am J. Obstet. Gynecol*., **230**(3S): S1089-S1106.

Kebede B and Shibeshi W (2022). *In vitro* antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. *Vet Med. Sci*., **8**(4): 1802-1815.

Kiokias S and Oreopoulou V (2021). A review of the health protective effects of phenolic acids against a range of

severe pathologic conditions (Including coronavirus-based infections). *Molecules*, **26**(17): 5405.

Kolekar K, Tambe S, Aderao R and Nayak C (2019). Chronic vulvovaginitis caused by *Candida dubliniensis* in an immunologically competent adult female. *Int. J. STD AIDS*., **30**(1): 90-93.

Le-Trilling VTK, Mennerich D, Schuler C, Sakson R and Lill JK (2022). Identification of herbal teas and their compounds eliciting antiviral activity against SARS-CoV-2 *in vitro*. *BMC biology*, **20**(1): 264.

Lee J, Cho JY, Lee SY, Lee KW, Lee J and Song JY (2014). Vanillin protects human keratinocyte stem cells against ultraviolet B irradiation. *Food. Chem. Toxicol*., **63**: 30-37.

Li X, Song J, Tan J, Zhang D and Guan Y (2024). Plant golden *C. sativus*: Qualitative and quantitative analysis of major components in stigmas and petals and their biological activity *in vitro*. *J. Pharm. Biomed. Anal*., **243**: 116115.

Lopes R, Costa M, Ferreira M, Gameiro P, Fernandes S, Catarino C, Santos-Silva A and Paiva-Martins F (2021). Caffeic acid phenolipids in the protection of cell membranes from oxidative injuries. Interaction with the membrane phospholipid bilayer. *Biochim. Biophys. Acta. Biomembr*., **1863**(12): 183727.

Lubbe A, Seibert I, Klimkait T and van der Kooy F (2012). Ethno pharmacology in overdrive: The remarkable anti-HIV activity of *Artemisia annua*. *J. Ethnopharmacol*., **141**(3): 854-859.

Luo X, Du C, Cheng H, Chen JH and Lin C (2017). Study on the anticoagulant or *Procoagulant* activities of type II phenolic acid derivatives. *Molecules*, **22**(12): 2047.

Nguyen W, Grigori L, Just E, Santos C and Seleem D (2021). The *in vivo anti-Candida albicans* activity of flavonoids. *J. Oral Biosci*., **63**(2): 120-128.

Obeid S, Alen J, Nguyen VH, Pham VC and Meuleman P (2013). Artemisinin analogues as potent inhibitors of in vitro hepatitis C virus replication. *PloS one*, **8**(12): e81783.

Polito F, Di Mercurio M, Rizzo S, Di Vito M, Sanguinetti M, Urbani A, Bugli F and De Feo V (2024). Artemisia spp. essential oils: From their ethnobotanical use to unraveling the microbiota modulation potential. *Plants*, **13**(7): 967.

Pop TD and Diaconeasa Z (2021). Recent advances in phenolic metabolites and skin cancer. *Int. J. Mol. Sci*., **22**(18): 9707.

Refaey MS, Abosalem EF, Yasser El-Basyouni R, Elsheriri SE, Elbehary SH and Fayed MAA (2024) Exploring the therapeutic potential of medicinal plants and their active principles in dental care: A comprehensive review. *Heliyon*, **10**(18): e37641.

Renner G (2021). Regulation of sun protection products in the EU. *Curr. Dermatol. Rep*., **55**: 266-281.

Sakagianni A, Koufopoulou C, Feretzakis G, Kalles D and Verykios VS (2023). Using machine learning to predict antimicrobial resistance-A literature Review. *Antibiotics*, **12**(3): 452.

Salih AM, Qahtan AA and Al-Qurainy F (2023). Phytochemicals identification and bioactive compounds estimation of artemisia species grown in Saudia Arabia. *Metabolites*, **13**(3): 443.

Sayre RM, Agin PP, LeVee GJ and Marlowe E (1979). A comparison of *in vivo* and *in vitro* testing of sunscreening formulas. *Photochem. Photobiol*., **29**(3): 559-566.

Siegel RL, Giaquinto AN and Jemal A (2024). Cancer statistics, 2024. *Cancer J. Clin*., **74**(1): 12-49.

Silverstein J, Goyal N and Tsai KK (2024). for the long haul: Management of long-term survivors after melanoma systemic therapy. *Curr. Oncol. Rep*., **26**(7): 804-817.

Suárez-Fernández C and García-Pola M (2024). Exploring the prevalence and risk factors of benign and premalignant oral lesions in an adult population from Northern Spain: A pilot study. *Quintessence Int.,* **55**(5): 412-419.

Tilley L, Straimer J, Gnadig NF, Ralph SA and Fidock DA (2016). Artemisinin action and resistance in *Plasmodium falciparum*. *Trends. Parasitol*., **32**(9): 682-696.

Timm S, Sun H and Huang W (2024). Photorespiration - emerging insights into photoprotection mechanisms. *Trends. Plant. Sci.,* **29**(10): 1052-1055.

Trifan A, Czerwińska ME, Mardari C, Zengin G and Sinan KI (2022). Exploring the artemisia genus: An insight into the phytochemical and multi-biological potential of *A. campestris* subsp. *lednicensis* (Spreng.) Greuter & Raab-Straube. *Plants*, **11**(21): 2874.

Wang B, Sui J, Yu Z and Zhu L (2011). Screening the hemostatic active fraction of *Artemisia annua* L. *In-vitro*. *Iran J. Pharm. Res*., **10**(1): 57-62.

Wang X, Chiu W, Klaassen H, Marchand A, Chaltin P, Neyts J and Jochmans D (2023). A robust phenotypic high-throughput antiviral assay for the discovery of rabies virus inhibitors. *Viruses*, **15**(12): 2292.

Xie Y, Yang W, Tang F, Chen X and Ren L (2015). Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. *Curr. Med. Chem*., **22**(1): 132-149.

Yaneva Z and Ivanova D (2020). Catechins within the biopolymer matrix-design concepts and bioactivity Prospects. *Antioxidants*, **9**(12):1180.