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Using of Expired Silymarin Medication as A Source of Biologically Active Compounds and Green Synthesis of Nanoparticles

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Abstract

The law demands that each drug manufacturers display the expiration date on drugs. The drug is likely to lose some potency after the expiration date, but it is not clear how much potency has been lost in the period after the expiration date. The antioxidant activity of silymarin was determined by various methods, such as the scavenging capacity of DPPH and ABTS•+, and compared with ascorbic acid to determine whether the antioxidant activity of silymarin still exists after its expiration date. TLC was used to separate silymarin compounds and ensure their purity. FTIR was used to measure the quality and quantity of silymarin and identify its compounds. Silymarin extract was used as a reducing agent, converting AgNO3 into AgNPs using magnetic stirring. The results indicated that silymarin has strong antioxidant activity that persists at high temperatures and an acidic pH. The study proved that even after silymarin's validity period, it can still maintain efficacy, stability, and biological activity. In conclusion, expired silymarin can be recycled by using simple techniques that enable the detection of the compounds they contain, the number of functional groups, and the presence (or absence) of their stability and activity.

Keywords: Silymarin expired drug; Chemical Constituents; Biological activities; Silver nanoparticles; Shelf-life time.

1. Introduction

Manufacturers use the expiration dates listed on the medications as a traditional measure to guarantee their quality. Numerous studies have shown that most medications remain stable and useful for a considerable amount of time after the manufacturer's labelled expiration date. These include the Continuous Federal Shelf Life Expansion Programme (SLEP), a European Study of 50 Different Drugs at least 20 Years Old, and numerous other studies on long expiry dates [1].

Before 1979, medical manufacturers were not compelled to list the expiration date on medications or drugs. The Food and Drug Administration (FDA), a US government agency, in 1979, the law first made it necessary for all medical manufacturers to state the day on which the drug's full potency and security were evaluated. As a result, there is an additional opportunity for discarding medicines and drugs with expiration dates indicated on their packages. This resulted in significant losses for all purchasers of medicines and drugs, particularly for the US Army and health government departments, which were forced to replace the unused and expired medicines and drugs with fresh stock. When faced with the responsibility of crushing and replacing the stored expired medications, which were valued at more than a billion dollars, the US Army authorities took notice of this loss in 1985. This activated the method of testing and evaluating the viability and safety of the expired medications and drugs that was assigned to the FDA by the US Army [2].

The expiration date marks the end of the medication's shelf life and the active ingredient's lifetime. A medication's or drug's actual shelf life could be significantly greater than its stated expiration date. In some instances, the FDA will extend the expiration date of certain stockpiled ingredients when they are properly stored and extensively tested because the cost of replacing these stockpiles would add significantly to the yearly federal drug budget. Regardless of whether the deadline can be extended, doing so would not be in the best interest of the pharmaceutical business given that it would probably result in decreasing medicine and drug sales [3].

Based on scientific testing of the medication or drug, the shelf-life time of a medication or drug is the period of time known to stay within acceptable specifications for potency, effectiveness, and safety. The expiration date is the day on which a batch's lifespan ends. This also indicates the point at which its efficacy begins to wane or the hazardous level can start to exceed the desirable level [2,4]. The drug's lower potency level is commonly set at 90% of the specified label promise. The expiration date is determined by the storage conditions.

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Because not all pharmaceuticals decompose at the same rate, using a product after it has passed its expiration date may simply lead to a reduced amount of active components than specified on the label [1].

The expiration dates on prescriptions, pharmaceuticals, and supplements are a cautious estimate by the producers to ensure quality, according to the Harvard Medical School (HMS) Family Health Guide website. Medication that has passed its expiration date is usually still safe, but it might not be as potent or effective. FDA approval states that although the effectiveness of expired medications is reduced, they are still safe to use [5]. Producers add product expiration dates instead of doing that on scientific grounds for marketing reasons. They require change; having treatments or drugs on the shelf for 10 years is not advantageous to them [6].

The milk thistle, Silybum marianum, dried seeds, and fruits contain silymarin, a bioactive ingredient. It has well-established antioxidant and hepatoprotective activities in preclinical investigations [7,8,9]. It also acts as an anti-diabetic dietary supplement [10], and has a lipid-lowering effect [11,12]. Silymarin consists of flavonolignan isomers, such as silydianin, silycristin, isosilybin, and taxifolin, as well as the major ingredient silybin, or silibinin [13,14], which has cytoprotective activities and acts as a free radical scavenger and regulates enzymes involved with the development of cellular damage [15,16]. For more than two thousand years, people have used milk thistle as a traditional treatment for neurological disorders like Alzheimer's and Parkinson's disease [17].

Over the last decade, silymarin has gained a lot of interest as an herbal therapy for liver problems. The antioxidant properties of silymarin are thought to be responsible for many of its protective benefits [18,19]. In the gut, direct scavenging of free radicals and chelation of free Fe and Cu are the most efficient [20]. In stressful situations, it is critical to prevent free radical generation by inhibiting specific ROS-producing enzymes or increasing mitochondrial integrity [21].

Recent studies demonstrate that silymarin also plays a neuroprotective role in several neurological disorders (NDs). Silymarin's anti-inflammatory [22], antioxidant, anti-cancer, cardio-protective, radioprotective, and anti-apoptotic actions in biological systems, including model organisms and cell lines, are credited with these neuroprotective effects [23]. Silymarin may be a promising anti-fatigue and ergogenic aid, even though the precise bioactive phytocompounds and exact processes are still unknown [24].

The current work aims to evaluate the efficiency and activity of expired silymarin (Ex-S) compared with new unexpired silymarin (UnEx-S), in addition to determining their chemical constituents.

2. Experimental

2.1. Chemicals and reagents

Pure petroleum ether, chloroform, ethyl acetate, methanol, and ethanol were purchased from the E. Merck Co. (Darmstadt, Germany). Both 2, 2-azino-bis (ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were bought from Sigma-Aldrich (St. Louis, MO, USA). Silymarin (S) and butylated hydroxyl toluene (BHT), purchased from Sigma-Aldrich (St. Louis, MO, USA). Each capsule of the silymarin 140 mg, with an expiration date of September 2022, includes 175 mg of milk thistle seed extract, 40 mg of essential phospholipids, and 140 mg of silymarin.

2.2. Silymarin extraction

Silymarin capsules that had been collected and began to expire for 3 months were ground into a powder. According to Wianowska and Wiśniewski [25], the dried powder (20g) was submitted to extraction procedures using 100% ethanol. The organic solvent extract was mixed and concentrated at 40°C under vacuum to create a dry crude extract of ethanol.

2.3. Fractionation of ethanolic silymarin extract

Using petroleum ether as the solvent, 150 g of silica gel (60–120 mesh for column chromatography) was placed into the chromatographic column (40 cm long, 2.5 cm in diameter). Before being topped off with the packed column, five grams of ethanolic crude silymarin extract were blended, purified, and mixed with silica gel powder. The column was then repeatedly eluted with 100% petroleum ether, its polarity increasing by 20% between each mobile phase mixture, chloroform, and ethyl acetate solvent (a total of 11 fractions were formed, as shown in Table 1), and finally, the solvent was changed to acetone [26].

Table 1 Fractions obtained from silymarin ethanoic extract

Solvents	Fractions No.										
	1	2	3	4	5	6	7	8	9	10	11
Petroleum ether	100	80	60	40	20	0	0	0	0	0	0
Chloroform	0	20	40	60	80	100	80	60	40	20	0
Ethyl acetate	0	0	0	0	0	0	20	40	60	80	100

2.4. Thin layer chromatography (TLC)

Precoated silica gel plates (TLC F254) were used for the separation of active chemicals from expiration and non-expiration silymarin in addition to column fractions (11 fractions). Chloroform and acetone were used as the mobile phases at various ratios (9.5:0.5 v/v, 8:2 v/v, and 5:5 v/v). According to the Ciura *et al.* [27] procedure, two-dimensional TLC was performed for the separated spot to validate the presence of a pure compound.

2.5. Bio-autography for Antioxidant Activity

Using DPPH solution as a spray reagent, a quick TLC screening method to evaluate antioxidant activity was carried out. As previously described by Nair *et al.* [28], TLC was carried out for silymarin extracts (for expiration and nonexpiry) with the addition of semi-purified fractions (11 fractions). After drying, 0.2% DPPH in methanol was sprayed over TLC plates to measure the antioxidant activity. Antioxidant compounds are present when yellow or white spots appear against a purple background.

2.6. Antioxidant activity

2.6.1. DPPH radical scavenging activity

The method of Nithiya and Udayakumar [29] was used to examine the scavenging abilities of silymarin drug extracts and fractions. 2.0 mL of 0.16 mM DPPH solution (in methanol) was added to a test tube containing a 1.0 mL aliquot of the sample (extracts, fractions, and Ag-Nps-Eth) at 100 and 200 μgmL^{-1}. After being vortexed for a minute, the mixture was left at room temperature for 30 minutes in complete darkness. At 517 nm, the absorbance of each sample solution was measured against a blank (methanol), which included silymarin and ascorbic acid as a standard antioxidant. The following formula was used to compute the scavenging activity percentage (%):

$$\% \text{ Antioxidant activity} = [(A_{control} - A_{sample} / A_{control})] \text{ X } 100$$

Where: $A_{control}$ is the absorbance of the DPPH solution while A_{sample} is the absorbance of the sample.

2.6.2. ABTS radical cation scavenging assay

The $ABTS^{\bullet+}$ assay measured a substance's capacity to scavenge radical cations of 2,2-azino-bis-ethylbenzthiazoline-6-sulfonic acid (ABTS). The radical cation was created by combining 2.45 mM potassium persulfate (1/1, v/v) with 7 mM $ABTS^{\bullet+}$ stock solution. The mixture was then allowed to sit for 4–16 hours until the reaction was complete and the absorbance remained steady. According to Salla *et al.* [30], the $ABTS^{\bullet+}$ solution was diluted with ethanol until it yielded an absorbance of 0.700 0.05 at 734 nm for measurements. Following a 45-second mixing period, the photometric assay was run on 0.9 mL of $ABTS^{\bullet+}$ and 0.1 mL of the tested samples at concentrations of 100 and 200 μgmL^{-1}. After one minute, measurements were taken at 734 nm.

$$E = [(Ac-At)/Ac] \times 100$$

where At and Ac are the respective absorbances of tested samples and $ABTS^{\bullet+}$ in comparison with silymarin and ascorbic acid standards.

2.7. Preparation of expired silymarin silver nanoparticles (Ag-Ex-S-NPs)

At room temperature, 100 mL of a 1 mM $AgNO_3$ aqueous solution was stirred while 10 mg of expired silymarin ethanolic extract was directly dissolved in the mixture. KOH was used to raise the obtained solutions' pH level to 10. The reaction mixture was then heated to 70°C continuously for 30 minutes while being kept on a magnetic stirrer. The colour of the solution changed soon after the reaction started, allowing for the visual inspection of the reduction of Ag^+ ions to silver nanoparticles. Before further investigation, the nanoparticles were repeatedly centrifuged at 20,000 x g for 30 min [31]. Nanoparticles were then washed with sterile distilled water.

2.8. Characterization of Ag-Ex-S-NPs

2.8.1. UV–vis spectrophotometric analysis

The changing colour of the reaction medium was first observed by periodic reaction solution sampling, and then its UV-VIS absorption was calculated. The reaction mixture aliquots were analysed using a Jenway UV-Visible spectrophotometer in the 200–800 nm range, according to Khattak *et al.* [31].

2.8.2. Fourier Transform Infrared (FTIR) spectroscopy

Using a Shimadzu Fourier transform infrared spectrometer at room temperature, an FTIR (400−4000 cm−1) at a resolution of 3 cm^{-1} investigation of Ag-Ex-S-NPs in KBr pellets was performed [32].

2.9. Thermal and pH stability of expired silymarin extract

The antioxidant activity was measured before and after one mL of the expired silymarin extract was exposed to 100ºC for 60 min. The pH change of silymarin that has expired is used to assess the impact of pH. In order to determine whether or not expired silymarin was affected by pH, acidic, neutral, and basic pHs were created by adding HCl and/or NaOH [33].

2.10. Statistical analysis

The analysis of values uses means and SE. The Costat Statistics computer programme was used for the statistical analysis. A one-way analysis of variance (ANOVA) was used to construct the statistical analysis, which was then followed by the least significant difference (LSD) at P 0.05.

3. Results and Discussion

3.1. Detection of antioxidant activity of expired silymarin using TLC

The most important question that came to mind was: did the expired drug lose partially or completely its antioxidant activity after the expiration date, or did it still retain it? To answer this question, extraction of the expired silymarin (Ex-S) by absolute ethanol was performed, then applied to TLC together with the unexpired silymarin (Un-EX-S) for comparison.

The obtained results showed that both samples were approximately similar not only when seen under an ultraviolet lamp at 254 nm but also when the plates were sprayed with DPPH, as shown in Fig. 1. This means that the Ex-S still has the same antioxidant activity and active components as the Un-Ex-S. This result coincided with those published by Puzanova *et al.* (2019) [6] concerning the expired paracetamol-containing drug. The determination of the percentage of the retained antioxidant activity of the Ex-S was performed using a DPPH scavenging assay.

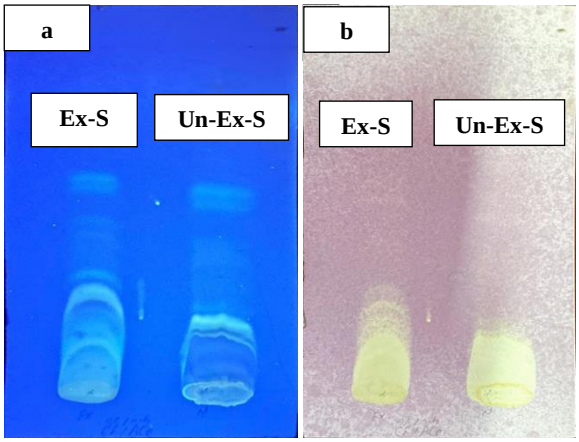

Fig. 1. The thin layer chromatography of Ex-S and Un-Ex-S, (a) under UV short wavelength (b) after sprying with DPPH

The confirmation of this result was noticed by using two extract concentrations (50 and 100 µg/mL) with the scavenging DPPH assay. Results recorded in Table 2 showed no significant differences between the antioxidant efficiency of both Ex-S and Un-Ex-S at the two concentrations used, and the activity is closely similar to

that of the natural antioxidant standard Ascorbic acid. These results showed agreement with those reported by Salman *et al.* [34], who proved that treatment with silymarin or ascorbic acid relieved acrylamide-induced hepatotoxicity in rats. Additionally, total protein and albumin levels in the blood, as well as HDL, ALT, and AST levels, decreased in Japanese quail. Plasma's total antioxidant capacity was greatly enhanced by using milk thistle levels of 0.5% and 1.5%, according to Khazaei *et al.* [35]. Silymarin can be used as a hepatoprotective drug based on the clinical evidence that is now available because of its strong antioxidant [36], antifibrotic, and anti-inflammatory effects. Silymarin is advised for usage in chronic liver disease in the recommendations approved by the Russian Medical Scientific Society of Therapists and the Gastroenterology Scientific Society of Russia because of its well-established role in lowering oxidative stress [18].

Table 2. Scavenging activity (%) of Ex-S and Un-Ex-S compared with ascorbic acid as natural antioxidant standard against the DPPH radical

Extracts	Concentration (µg /mL)		IC_{50} (µg /mL)	Retention of activity (%)	
	50	100		50	100
Ex-S	66.57^{b}±0.48%	81.00^{b}±0.40%	36.84	100	100
Un-Ex-S	66.30^{b}±0.13%	80.41^{b}±0.62%	37.06		
Ascorbic acid	78.57^{a}±0.29%	83.14^{a}±0.18%	29.91		
LSD (0.05)	0.66	0.88			

Values are mean ± SE (n=3). The mean values within a column indicate significant differences ($p<0.05$). LSD is the least significant difference.

3.2. Fractionation of the Ex-S ethanolic extract using column chromatography

Fractionation of the ethanolic extract was performed using silica gel-packed column chromatography and elution by a mobile system of pet. ether, chloroform, and ethyl acetate in different combinations (20% increased polarity between each mixture). Eleven fractions were plotted on TLC plates together with Ex-S and Un-Ex-S extracts to examine their antioxidant efficiency.

The results were first screened under a short ultraviolet lamp of 254 nm, then the spots were sprayed with DPPH reagent as an autobiography, confirming the antioxidant activity of expired silymarin as well as some of its fractions compared to the Un-Ex-S. Fractions no. 2, 3, 4, and 9 showed the best results compared to Un-Ex-S and were recorded in Fig. 2. The obtained results may be due to the polarity index of pet. ether (0.1), which allows the non-polar lipids (phospholipids) in the mixture to be dissolved, and to the polarity index of chloroform (0.259). So these solvents caused the best separation of the compounds in the tested samples. The obtained results were in agreement with the ones recorded by Parys *et al.* [37], who used similar TLC techniques for detecting the best solvent system for the separation of active compounds of diclofenac in enteric-coated tablets.

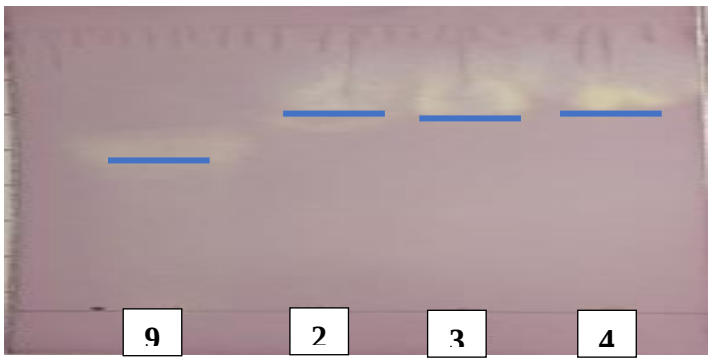

Fig. 2. TLC-bioautography of column fractions (2, 3, 4, and 9) after sprying with DPPH reagent

Different ratios of chloroform and acetone (9.5:0.5, 2:8, and 5:5 v/v) were examined in order to determine the most effective mobile system combination that results in the optimum separation of the active ingredients of the expiry silymarin using TLC plates and, subsequently, their antioxidant activity. When the TLC plates were sprayed with DPPH reagent, the active spots appeared yellow or white on a purple background, confirming the separation of the contained active ingredient under a UV lamp at 254 nm. The results were well illustrated in Fig. 3, where clear and accurate bands were noticed on TLC where fraction No. 1 was higher than those of 2 and 3. The most convenient mobile system combination for separation of active compounds was chloroform 9.5: acetone

0.5, which consequently induced the best qualitatively recorded antioxident activity on spraying by DPPH (auto-biography). These results are in parallel with those reported by Sridhar and Charles [38] for the *in vitro* antioxidant activity of Kyoho grape extract. The effective isolation of active ingredients appears to depend on the solvent and a method of extraction that are carefully chosen, both of which may help this precious plant material be used more successfully in a variety of businesses [39].

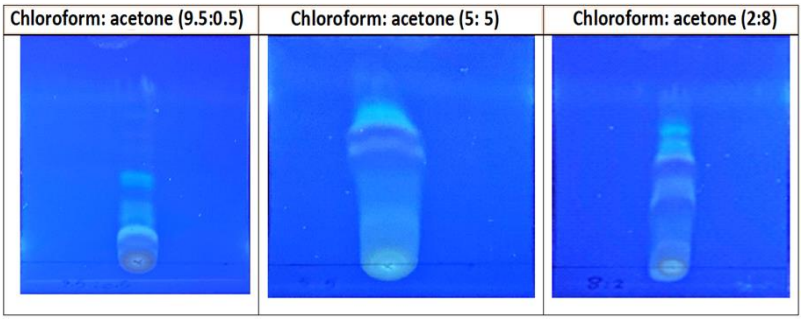

Fig. 3. TLC bio autography of Ex-S ethanoic extract under a short ultraviolet lamp at 254 nm

3.3. Thermal and pH stability of Ex-S ethanolic extract

Is this medication susceptible to changing pH levels and storage temperatures, or is it resistant to them? To experiment, the Ex-S extract was exposed to 100ºC for 60 minutes, after which its activity was assessed using the ABTS radical cation scavenging assay. As can be seen in Fig. 4, the acquired results showed a considerable drop in its activity (38±0.24%) compared to the untreated one (82±0.54%), which is more than half of the normal activity with retention activity 46.34%. This finding is consistent with research done by Zhang *et al.* [40] and may be explained by the fact that flavonoids are heat-sensitive. Heating to a temperature of more than 75ºC can kill the enzyme responsible for flavonoid production, which in turn reduces the antioxidant activity of the Ex-S extract.

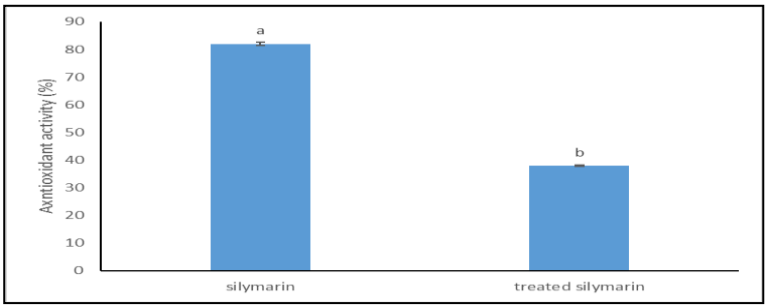

Fig. 4. The antioxidant activity (%) of treated Ex-S with 100ºC for 60 min using ABTS$^{\bullet+}$.

It was known that the suitable medium for silymarin is slightly acidic, equal to 6.6, so at pH 4, its antioxidant activity using the ABTS radical cation scavenging assay decreased to 66.66±0.82% compared to the untreated silymarin (82±0.54%) with retention activity 81.29%, while elevated pH to 7 and 10 induced a sharp drop in antioxidant efficiency to 25.33±0.26 and 14.66±0.18%, respectively, (with retention activity 30.89 and 17.88%, respectively) as indicated in **Fig. 5.** So, the best conditions for storing and conserving silymarin are to store it at a controlled temperature (20–25ºC) and an acidic pH.

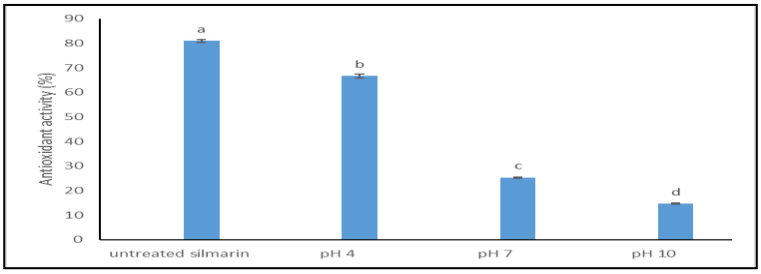

Fig. 5. The antioxidant activity (%) of Ex-S at different pH degrees (4, 7, and 10) using ABTS$^{\bullet+}$ assay

3.4. Green biosynthesis and characterization of silver nanoparticles from the Ex-S ethanolic extract

Many diseases have been found to benefit from the use of antioxidants as preventative and therapeutic activity. Unfortunately, due to their limitations, which include low permeability, poor water solubility, instability during storage, and gastrointestinal degradation, these antioxidants have had very little success up to this point. Together, material sciences and nanotechnology have significantly enhanced and decreased the generation of free radicals during the manufacturing of nanoparticles in several fields; the resulting nanoparticles are known as nano-antioxidants.

As Ex-S tablets still have a large percentage of their antioxidant activity and contain four active phytoconstituents, they may serve as reducing agents for silver ions (Ag^{+}) to silver nanoparticles ($Ag^{\bullet}$). This was achieved by using $AgNO_3$ at pH 7 at a constant temperature of 70 and stirring for 30 minutes. The highly antioxidant activity (or reducing power) of silymarin was compared with the natural antioxidant standard (ascorbic acid) as mentioned in Table. 2. The obtained results reported that the silymarin has high antioxidant activity near to the activity of ascorbic acid by 81 and 83 % respectively, and this may be due to its chemical structure as a polyphenolic structure which contains major group that help the compound to scavenge the ROS or reduce different oxidizing agent; these major chemical groups are: a- phenolic hydroxyl group, which can donate hydrogen atoms to neutrilize free radicals; b- conjugated system which allow for electron delocalization, stabilizing the free radicals formed after neutrilization; c- Benzodioxane ring which enhances the ability to scavenge reactive oxygen species. So, these structural features make silymarine a have high ability for redcing the silver ions and converted it to silver atoms. In addition to the ability of silymarin compounds as antioxidant to protect cells and tissues from oxidative stress and associated damage. These results were in agreement with the results reported by Surai [41] who mentioned that the possible antioxidant mechanisms of silymarin compound are one of the following: a direct scavenging free radicals; chelating free heavy metals; preventing free radical formation; maintaining an optimal redox balance in the cell.

3.4.1. UV-VIS spectrophotometric absorption

The visual color change of the reaction mixture indicated the possible formation of AgNPs, which, on centrifugation and washing, were subjected to ultraviolet spectrophotometric absorption, according to Khattak et al. [31]. Silver nanoparticles showed a maximum absorbance of 1.9 at 300 nm (Fig. 6), while a minimum absorption of 0.04 was determined at 800nm. The resulting absorption spectra of AgNPs displayed an evident resonance band maximum at 300 nm, which is a good indicator of silver nanoparticles formation [42].

It is well known that the size and shape of the silver nanoparticles reflects the absorbance peak. The peak shifts to longer wavelengths with increase in particle size. At the beginning of reaction the intensity was high at 300 nm,the range was increasing up to 4h hour 503 nm, while in 24 hour the intensity was 795 nm.

This means that as the wavelength decreased, the activity of the nanoparticles (as antioxidants) increased. These results coincided with those obtained by Zhao *et al.* [43], who proved the high antioxidant activity of nanosilver through the emulsion solvent evaporation method. Such environmentally friendly nanoparticles in antibacterial, wound healing and other medical and electrical applications make this approach potentially promising for the large-scale synthesis of other inorganic nanomaterials [44]. Moreover, comparing synthetic Ag-NPs-Me to methanolic extract, the antioxidant activity increased [45].

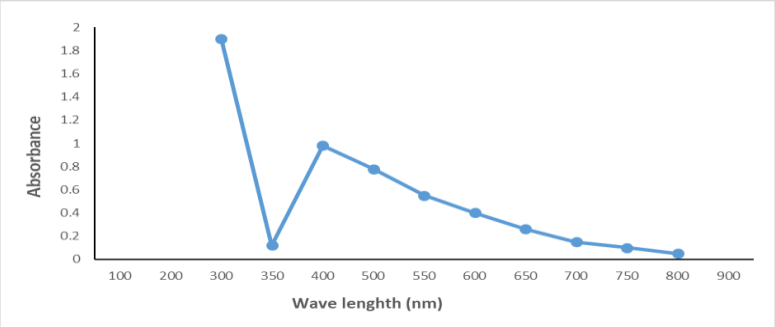

Fig. 6. UV–VIS spectra of Ag-NPs-Ex-S

3.4.2. Antioxidant activity of AgNPs synthesized by Ex-S ethanolic extract

As demonstrated in Fig. 7, the antioxidant capacity of Ex-S ethanolic extract and the AgNPs biosynthesized were compared. The nanoparticles' activity was only about half (45±0.81%) that of Silymarin's (82±0.58%).

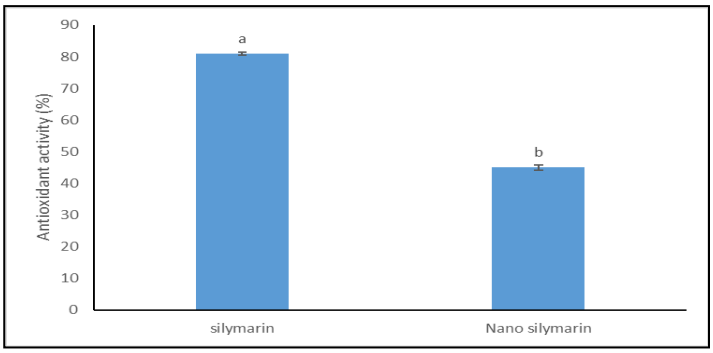

Fig. 7. The antioxidant activity of silymarin and Ag-NPs-Ex-S using the $ABTS^{\bullet+}$ assay

According to the results, AgNPs showed reduced antioxidant activity compared to the ethanol extract of EX-S. The suggested reason is that the phenolic and flavonoid components in the ethanol extract were consumed in the reduction process in the green synthesis of the AgNPs [46].

For determining the functional groups of compounds contained in Ex-S that were involved in the reduction of silver ions to silver nanoparticles, Fourier transform Infrared spectroscopy (FTIR) was applied for both the Ex-S extract and the AgNPs-Ex-S.

The results, recorded in Fig. 8 and Table 3, showed the presence of slight shifts in the FTIR peaks of expiry silymarin extract (3354, 2927, 1642 cm^{-1}) and the biosynthesized AgNPs (3354, 2973, 1640 cm^{-1}). The peaks suggested the involvement of some functional groups in the reduction of silver ions as well as acting for capping and stabilising its nanoparticles (OH, CH_3, CH_2, C=C). Fig. 8 indicated that there was vibrations at wave length below 500 cm^{-1}, which is a finger print of AgNPs formation [44].

It is known that hydroxyl groups (O–H) possessed a great ability to bind with silver ions (Fig. 9) [46]. According to our FTIR results, the antioxidant activity of silver nanoparticles, including phenolics or other reducing components, may occur in conjunction with the reduction of silver ions, which could be the possible mechanism of AgNP synthesis.

Table 3. Wavenumber range of characteristic bands and corresponding assignments for Ex-S and Ag-NPs-Ex-S

Wavenumber (cm^{-1})	Fraction groups assigned	Ex-S extract	Ag-NPs-Ex-S
2300 – 4000	Polymeric hydroxyl compound O – H stretching	2354	3354
2900 – 3000	Methyl and methylene group	2927	2973
1650	C=C	1642	1640

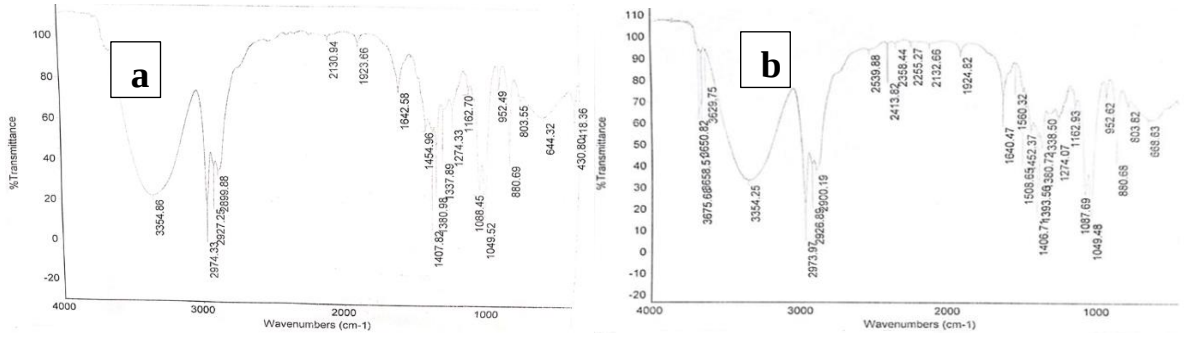

Fig. 8. FTIR spectra of Ex-S ethanolic extract (a) and its synthesized AgNPs (b)

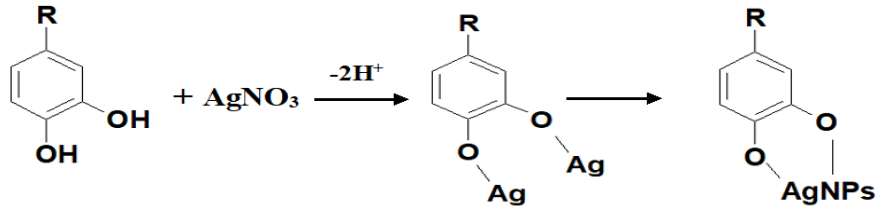

Fig. 9. Suggested reduction mechanism of Ag^+ to Ag^0 by phenolic compounds according to Ghasemi *et al.* [46].

4. Conclusions

In vitro tests, ABTS radical scavenging, and the Folin-Ciocalteau reaction were used to validate the extracts' significant antioxidant activity. Taxifolin content in extracts have a relationship to acetylcholinesterase inhibition, and flavonolignans have a particularly strong anti-inflammatory and antioxidant effect [14,47]. This study concluded that an extension of the expiration date of a drug, utilizing innovative stability tests, would help reduce the annual volume of expired medication. The active ingredients in expired silymarin drugs are still active and can compete with the unexpired ones in efficiency. Increasing awareness through educational programs about proper disposal guidelines is necessary for controlling medication waste. Future studies will be required to investigate this important issue of medication waste and how to tackle it. Therefore, it may be better to prevent this requirement by teaching the public and attracting doctors and chemists to recognise their roles in both the cause of the problem and its solutions.

5. Conflicts of interest

There are no conflicts to declare.

6. References

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