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Utilization of *Saussurea costus* Powder as Antioxidant, Antimicrobial Agent, and Anti-Alzheimer's (*In vitro study)*

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Abstract

Saussurea costus (S. costus) is an Indian medicine utilized to treat several diseases. It has a variety of pharmacological properties, including antioxidant activity, and contains phytochemical compounds. The goal of this study is to explore the antioxidant activity, antimicrobial, and anti-Alzheimer's activity of *S. costus*. According to the results, *S. costus* root powder recorded the following contents: moisture, ash, protein, fat, and total carbohydrates, which were 3.90, 4.38, 6.60, 5.98, and 79.14 %, respectively. Total phenols were detected in greater quantities than total flavonoids (254.86 mg GAE/100g and 161.20 mg QC/100 g, respectively), and the DPPH scavenging radical value was 88.84%. The main flavonoid component was Naringin (14.58 mg/100g), while Ellagic acid was the primary phenolic acid (14.83 mg/100g). *S. costus* was highly effective as an antimicrobial due to the presence of these components. In addition, the root powder exhibited minimal toxicity against the Vero cell, with an IC_{50} of 70.50±3.17 µg/ml value. The results demonstrated that the inhibition percentage of the acetylcholinesterase (AChE) enzyme associated with Alzheimer's disease increased with higher concentrations of *S. costus* root extract, which exhibited IC_{50} of 155.3±10.28 µg/ml compared to the control (Donepezil standard**)**. Partial substitution of wheat flour with 1 to 4% *S. costus* powder enhanced the antioxidant activity of crackers by increasing polyphenolic compound content and nutritional value while maintaining sensory quality and consumer acceptability. Furthermore, the low total microbiological count observed in the cracker formulations during the three-month storage period highlights the natural antioxidant and antimicrobial efficacy of *S. costus* powder. Given the complex, multifactorial etiology of Alzheimer's disease, this root powder represents a safe and health-promoting candidate for the future increase of disease-modifying therapies targeting Alzheimer's.

Keywords: *Saussurea costus;* Anti-Alzheimer; Functional properties; Cytotoxicity; Acetylcholinesterase inhibitor.

1. Introduction

Medicinal plants contain active phytochemicals that can be utilized as natural alternatives to chemical food preservatives. The compounds have antiviral, antibacterial, and antifungal properties that inhibit the growth of pathogens and spoilage microbes **[1, 2]**. Medicinal plants contain a range of bioactive compounds like alkaloids and flavonoids, which can be easily available sources for safer food preservation methods. The use of such natural compounds could improve food preservation along with reducing health risks from synthetic chemicals **[3,4].** Among these medicinal plants is *Saussurea costus* (commonly known as "Al-Kost Al-Hindi" in the Arab world), which traditional healers have utilized since the Islamic era. *S. costus* has been used as a sedative, antiseptic, bronchodilator, stimulant, and mucus repellant **[5]**.

Numerous studies on the biological activity of *S. costus* roots, which are also known as *S. lappa*, have shown that they have anti-trypanosomal properties **[6]**. Additionally, *S. costus* holds a significant position in traditional Chinese medicine, where it has been valued for its therapeutic properties and diverse applications **[7]**. It is recognized as a prolific source of many bioactive phytoconstituents, such as flavonoids, phenylpropanoids, lignans, coumarins, sesquiterpene lactones, steroids, and volatile oils, which demonstrate a range of pharmacological effects **[8]**. It comprises substances termed "complement inhibitors" that are constructive in

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the treatment of diseases associated with the excessive stimulation of the complement system, such as rheumatoid arthritis, respiratory distress, and systemic lupus erythematosus **[9].** *S. costus* ethanolic extract reduces oxaliplatin-induced testicular damage in rats due to its anti-inflammatory and antioxidant properties**. [10]** Cell lines examination of *S. costus* has demonstrated strong anticancer properties **[11]**. An ethanol extract from *S. lappa* (synonymous with *S. costus*) has shown a varied antibacterial activity against various bacterial pathogens **[12]**. Numerous investigations have documented the presence of phytochemicals qualities in *S. costus* roots that have antiviral, anti-inflammatory, anti-ulcer, and anti-immune properties **[13]**. The development of treatments for Alzheimer's disease (AD) is one of the most significant challenges of the century. Alzheimer's disease (AD) symptoms and cognitive impairments are linked to cholinergic deficits caused by the degeneration of cholinergic neurons in the basal forebrain, along with senile plaques and neurofibrillary tangles formation **[2, 14]** Inhibiting acetylcholinesterase (AChE) is a key therapeutic strategy for managing AD. Beyond approved treatments, many plant-derived phytochemicals, such as flavonoids, alkaloids, and phenolic compounds, have shown AChE inhibitory activity. These natural compounds offer potential as complementary therapies for AD due to their dual roles in AChE inhibition and neuroprotection **[15]**.

The current study aims to evaluate and quantify phytochemical compounds existing in *S. costus*. Additionally, the study examined the antimicrobial activity of *S. costus* against selected pathogenic microorganisms. Furthermore, the cytotoxic activity and acetylcholinesterase (AChE) inhibitory potential of the extract were assessed in vitro to explore its possible therapeutic effects against Alzheimer's disease.

2. Materials And Methods

2.1. Materials

Saussurea costus (S. costus) was purchased from Medicinal and Aromatic Plant Research Department, Horticulture Research Institute, Agriculture Research Center (Giza, Egypt), and was stored in polyethylene bags at -18 °C until analysis.

Wheat flour with 72% extraction was purchased from the South Cairo Mills Company (Giza, Egypt). Additional components (sugar, baking powder, salt, and sunflower oil) were bought from the local market (Giza, Egypt).

Solvents and the Folin-Ciocalteu reagent were acquired from El-Nassr Pharmaceutical Chemical Co., Egypt, Quercetin, gallic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were acquired from Sigma Chemical Co. (St. Louis, Mo).

All tested bacteria (Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* RCMB010052, and *Salmonella typhimurium* ATCC14028; Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538 and Methicillin-resistant *S. aureus*); (*Candida albicans* RCMB 005003); and *Aspergillus niger* (isolated strain) were obtained from the Regional Center for Mycology and Biotechnology-Al-Azhar University (Cairo, Egypt)

Mammalian cell lines: Vero cells, originally isolated from the kidney cells of the African green monkey, were obtained from the Tissue Culture Unit at VACSERA. Chemicals and reagents used in the study—including fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamicin, 0.25% Trypsin-EDTA, and 1% crystal violet stain—were purchased from Sigma (St. Louis, MO, USA).

The enzyme acetylcholinesterase was acquired from Sigma-Aldrich from Electrophorus Electricus. Cat number: 3389. The substrate, acetyl thiocholine iodide, and the indicator (DTNB Ellman's reagent) were acquired from Sigma-Aldrich.

2.2. Methods

2.2.1. Chemical composition

The moisture, protein, ash, and fat contents of S. costus powder were evaluated. The moisture, protein, ash, fiber, and fat contents in substituting crackers, were also determened according to **AOAC [16]**. The difference was used to compute the carbohydrate content. Additionally, the calculation of **Mansour and Khalil [17]** was used for the total calorie (kcal) estimated as (Carbohydrate x 3.87) + (Protein x 4.02) + (Fat x 9).

2.2.2. Phytochemical analysis

The Folin-Ciocalteu (FC) reagent method was utilized to evaluate the total phenols in samples of *S. costus* root powder and its crackers with various formulas **[18].** The findings were expressed in mg of gallic acid equivalent (GAE) per 100 gm. The methods of **Kanatt *et al.* [19]** were used to determine the total flavonoid compounds, and the findings were expressed in mg as quercetin (QC) equivalent /100 gm).

2.2.3. Antioxidant activity

The antioxidant activity of *S. costus* roots powder and 1, 2, 3, and 4% cracker samples were measured using the approach described by **De Ancos *et al.* [20]** employing the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent. Absorbance was quantified at 517 nm utilizing a UNICO spectrophotometer (Model: UV 2000, UNICO Instruments Co., Ltd.). The antioxidant capacity was calculated as inhibition percent based on the following equation:

$$\% \text{ DPPH (antioxidant activity)} = [(Ac - As) \div Ac] \times 100\%$$

Where: Ac: Control sample absorbance and As: Test sample absorbance

2.2.4. Fractionations of phenolic and flavonoid compounds by HPLC

An HPLC Agilent (1200 series), performing a C18 reverse phase column (Zorbax ODS 5 µm, 4.6 x 250 mm) kept at 35ºC, an autosampler, a solvent degasser, an ultraviolet (UV) detector, and a quarter HP pump (series 1050), was used to estimate the polyphenols fractions. Chromatograms for flavonoids were acquired at 330 nm, and for assessed phenolic acids, at 280 nm. All components were identified and measured by comparing peak regions with external standards **[21]**.

2.2.5. Antimicrobial activity

Antimicrobial activity of *S. costus* against *P. aeruginosa* (ATCC 27853), *E. coli* (RCMB010052), and *Salmonella typhimurium* (ATCC14028) as Gram-negative bacteria, *Staphylococcus aureus* (ATCC 6538), Methicillin-resistant *S. aureus* (MRSA) as Gram-positive bacteria, *Candida albicans* RCMB005003 and *Aspergillus niger* isolated strain as fungi. Tests were conducted using 96-well flat polystyrene plates. 80 µl of lysogeny broth (LB broth) was combined with 10 µl of *S. costus* root powder extract (final concentration 30% w/v). Subsequently, 10 µl of bacterial culture solution (log phase) was introduced, and the plates were incubated at 37°C for 16–18 hours overnight. Subsequent to incubation, the beneficial antibacterial impact of the examined *S. costus* was evident as distinct zones in the wells. Conversely, compounds without antibacterial activity produced opaque wells attributable to bacterial proliferation. The untreated pathogen functioned as the control. The finding was measured at 600 nm using a Spectrostar Nano Microplate Reader (BMG LAB-TECH GmbH, Allmendgrun, Germany) after 20 hours. Antimicrobial activity and minimum inhibition concentration (MIC) were determined using the method described by **Balouiri *et al*. [22].**

2.2.6. Cytotoxic activity

2.2.6.1. Cell line Propagation

The cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with gentamicin (50µg/ml), 10% heat-inactivated fetal bovine serum, 1% L-glutamine, and HEPES buffer. Twice a week, each cell was sub-cultured and sustained at 37µL of bacterial culture solution (in log phase) was introduced, and the plates were incubated at 37°C overnight for 16–18 hours (a humidified environment) with 5% CO_2.

2.2.6.2. Cytotoxicity assay

The cells were cultured in 96-well plates containing 100 µl of growth media with 1×10^4 cells per well for the cytotoxicity assay. Following a 24-hour incubation period, a fresh medium containing varying concentrations of the test material was added. The microtiter plates were maintained at 37ºC in a humidified incubator with 5% CO_2 for 48 hours. Each test sample concentration utilized three wells. Control cells were cultured with or without DMSO and tested samples. After the incubation time, the media were removed, and a 1% crystal violet solution was introduced to each well (30 minutes). Subsequent to the removal of the stain, the plates were rinsed with distilled water to exclude any residual staining. Thirty percent glacial acetic acid was introduced to each well and thoroughly mixed. The plates were subsequently agitated on a Microplate reader (TECAN, Inc.). The absorbance was measured at 490 nm in accordance with **Osman [23]**

2.2.7. In vitro anti-Alzheimer activity

2.2.7.1. Sample preparation

The following concentrations were used to prepare the sample: 500, 250, 125, 50 and 25 µg/mL. Dissolving the samples initially in DMSO (Dimethyl Sulfoxide) and diluting them to the desired concentrations with water will ensure that the final concentration of DMSO does not exceed 1%.

2.2.7.2. Donepezil standard preparation

Standard donepezil was prepared at the following final concentrations in methanol: 0.416, 0.0416, 0.00416, 0.000416, and 0.0000416 µg/mL.

2.2.7.3. Anti-Alzheimer's assay

According to **Osman *et al.* and Elmann *et al.* [23, 24],** with some modifications. In summary, 10µL of the indicator solution (DTNB Ellman's reagent) at a concentration of 0.4mM dissolved in buffer (1): 100 mM Tris buffer at pH 7.5 was added to a 96-well plate, followed by the addition of 20 µL of enzyme solution (acetylcholinesterase at a final concentration of 0.02 U/mL in buffer (2): 50 mM Tris buffer at pH 7.5 with 0.1% bovine serum albumin). Then, 20 µL of the *S. costus*/standard solution was added, followed by adding buffer 1(140µL). The mixture was allowed to stand for 15 minutes at ambient temperature. Subsequently, 10 µL of the substrate (acetylcholine iodide buffer 1 (0.4mM) was promptly added to each well. The plate was incubated in the dark for 20 minutes at ambient temperature. After incubation time, the color was assessed at 412 nm. Data were expressed as means ± standard deviation (SD).

Data were recorded using the FluoStar Omega microplate reader. The IC_{50} value was found by converting the concentrations to their logarithmic values and utilizing the non-linear inhibitor regression equation (log (inhibitor) vs. normalized response – variable slope equation) using GraphPad Prism 6®, after data processing in Microsoft Excel®.

2.2.8. Cracker preparation

The crackers were prepared following the method described by **Han *et al.* [25]** with some modifications. *S. costus* powder was substituted instead of 1, 2, 3, and 4% wheat flour, and the recipe was as follows:

One hundred grams of wheat flour (72% extraction), 25 grams of sunflower oil, 3 grams of sugar, 3 grams of salt, 3 grams of baking powder, and an appropriate amount of water were used to prepare the dough. In a dough mixer, oil, sugar, salt, and water were combined for one minute with a flat beater, then scraped down and mixed for more than three minutes at a high speed. After gradually adding the dry ingredients of soft wheat flour (or blends) and baking powder to the mixture and mixing it on a low speed for three minutes, the resulting dough was allowed to rest for five minutes before being sheeted to a thickness of three millimeters. Dough pieces with an outer diameter of 5 mm were shaped using templates. The crackers were baked at 170°C for 15 minutes and then allowed to cool at room temperature for one hour before evaluation.

2.2.9. Sensory evaluation

In a laboratory preference test, twenty panelists from Food Technology Research Institute, Agriculture Research Center (Giza) evaluate crackers and other flour-based crackers. Using a ten-point hedonic scale, where one denotes a strong dislike, and ten denotes a strong like, they assessed the crackers according to their color, puffing, taste, crispiness, texture, and overall score **[26].**

2.2.10. Microbial assay

Five samples of crackers were tested at zero time and after three months of storage, recording the total number of bacteria, yeast, and mold. Conducted on the control, 1, 2, 3, and 4% samples to determine the microbial analysis as stated by **AOAC [16].**

2.2.11. Statistics analysis

SPSS 16.0 was used to examine the analytical data. Descriptive statistics were used to calculate means and standard deviations. Multiple range tests and analysis of one-way variance (ANOVA) were used to compare samples. The statistical significance was set at $P \leq 0.05$.

3. Results And Discussion

3.1. Chemical and phytochemical analysis of *S costus* roots powder

The proximate chemical composition of *S. costus* roots powder is shown in Table 1. In general, the composition of *S. costus* roots was 3.90, 4.38, 6.60, 5.98, and 79.14 % for moisture, ash, protein, fat, and total carbohydrates, respectively. **Akl and Younos [27]** found that *S. costus* contains carbohydrates and protein (83.74 and 7.43%, respectively). Meanwhile, ash, moisture, and fat were 3.93, 0.70, and 4.20%, respectively.

Allam and Amin [28] examined the chemical component values of *S. costus* root powder and found that they were 4.37g/100g protein, 1.51g/100g ash, 1.14g/100g fiber, 83.30g/100 g carbs, and 9.57g/100 g fat.

As presented in Table 1, *S. costus* contains higher total phenolic content compared to total flavonoid content, with values of 254.86 mg GAE/100g and 161.20 mg QC/100g, respectively. In addition, the root powder of *S. costus* demonstrated significant antioxidant activity, with a DPPH radical scavenging capacity of 88.84%. This may be caused by the impact of phenolic and flavonoid contents on antioxidant activity. These results were parallel to **Premalatha and Lakshmi, [29]** who investigated the phytochemicals of *S. costus* ethyl acetate extract, which showed a notable level of total phenolic and flavonoid components. Meanwhile, **Hashimi *et al.* [30]** discovered that methanolic *S. costus* roots extract recorded the highest values of flavonoid and phenolic contents, ranging from 16.2 to 67.60 mg QE/g and 12.34 to 75.02 mg GAE/g, respectively. Also, **Al-Zayadi *et al.* [31]** indicated that *S. costus* aqueous extract was rich in phenolic compounds, which exhibited potent antioxidant and anti-scavenging properties.

Table 1: Chemical composition and phytochemical of *S. costus* roots powder

Chemical composition	*S. costus* (g/100g)	Phytochemical	*S. costus*
Moisture	3.90±0.01	**Total phenols (mg GAE/100g)**	254.86 ±0.22
Ash	4.38±0.02	**Total flavonoids (mg QC/100g)**	161.20 ± 1.32
Protein	6.60±0.03	**DPPH scavenging activity (%)**	88.84 ±0.9
Fat	5.98±0.003		
***Total Carbohydrate**	79.14±0.01		

*Total carbohydrate =100 - (Moistuer + Ash + Protien + Fat).

3.2. Fractionation of *S.costus* roots phenolic and flavonoid compounds by HPLC

The data in Table 2 reveal the phenolic and flavonoid compounds identified in the *S. costus* using HPLC. The HPLC analysis of the *S. costus* extract revealed 24 distinct peaks, representing two major categories of phytochemicals compounds: flavonoids and phenolics.

The results indicate that the major flavonoid compounds were Naringin, Acacetin 7 neo-rutinoside, and Kaemp.3-(2-p-comaroyl) glucose (14.58, 9.35, and 7.26 mg/100g respectively) followed by Quercetrin, Naringenin, Apigenin, Quercetin, and Rutin (4.58, 4.44, 4.35, 3.34, and 3.25 mg/100g respectively). In addition, Kampferol, Apigenin-7-glucose, and Rosmarinic were the lowest flavonoids. In contrast, the major phenolic compounds identified were Ellagic acid, Catechol, Catchein, Pyrogallol, and Chlorogenic acids (14.83, 11.09, 10.81, 10.12, and 8.14 mg/100g, respectively), followed by *P*-OH- benzoic acid, Caffeic acid, Caffeine, and Ferulic acid (4.74, 2.63, 1.53 and 1.26 mg/100g, respectively). Meanwhile, 4-Amino-benzoic, Coumarin, Gallic, and Vanillic acids were the lowest components. These results are in the same line with **Ashry [32],** who identified the phenolic compound of *S. costus* ethanolic extract by HPLC. The study revealed that the extract contained high levels of naringenin, chlorogenic acid, ferulic acid, taxifolin, gallic acid, and caffeic acid as the major phenolic compounds. **Al-Zayadi *et al.* [31]** identified two flavonoids (kaempferol and rutin) and two phenolic acids (catechin and apigenin) in the *S. costus* roots ethanolic extract.

Table 2: Fractionation of *S. costus* roots phenols and flavonoid components

Phenols	mg/100g	Flavonoids	mg/100g
Pyrogallol	10.12	Rutin	3.25
Gallic acid	0.73	Naringin	14.58
Catechol	11.09	Rosmarinic	0.19
4-Amino-benzoic acid	0.89	Quercetrin	4.58
Catechin	10.81	Apigenin-7-glucose	1.08
Chlorogenic acid	8.14	Quercetin	3.34
P-OH- benzoic acid	4.74	Naringenin	4.44
Caffeic acid	2.63	Kaemp.3-(2-p-comaroyl) glucose	7.26
Vanillic acid	0.71	Kampferol	1.91
Caffeine	1.53	Acacetin 7 neo.rutinoside	9.35
Ferulic acid	1.26	Apigenin	4.35
Ellagic acid	14.83		
Coumarin	0.86		

3.3. Antimicrobial activity of *S. costus* roots

Data presented in Figure 1 and Figure **2** showed the antimicrobial activity of *S. costus* root extract against all tested pathogen microorganisms. The positive effect was shown against *P. aeruginosa*, *E. coli*, *Salmonella typhi, S. aureus,* and *MRSA* as antimicrobial activity. *S. costus* root extract also had a positive effect against *C. albicans* and *A. niger*, similar to control (untreated pathogens). *S. costus* extract showed the maximum activity against *E. coli* (2.291 OD), whereas *S. typhi* (0.729 OD) showed the lowest activity.

The minimum inhibitory concentration (MIC) is the lowest concentration of the tested material, which inhibits the growth of microorganisms within 24 hours. The MIC of *S. costus* roots against tested microbes (see **Table 3**) shows that the extract of *S. costus* roots extract had a low MIC (62.5 µg/ml) for *E. coli* and MRSA inhibition activity. However, certain microbial strains, including *P.aeruginosa*, *S. typhi*, and *S. aureus*, required relatively high concentrations of up to 250 µg/ml to limit bacterial growth effectively. In contrast, fungal strains such as *C. albicans* and *A.niger* required 200 µg/ml and 100 µg/ml, respectively, to inhibit fungal growth.

This antibacterial activity might be related to *S. costus* highest polyphenol content and the presence of catechin in a large amount, as mentioned previously. Polyphenols are recognized for their antifungal and antibacterial effects. *S. costus* exhibits superior antibacterial efficacy against gram-positive bacteria relative to gram-negative bacteria, attributable to differences in cell wall structure; *E. coli* and *P. aeruginosa* possess an additional outer membrane comprised of lipopolysaccharides, which serves as a barrier to numerous antimicrobial agents **[33**]. According to **Gutiérrez-Venegas *et al.* [34]**, catechin is an antimicrobial polyphenol that works against a range of bacterial species, including *Streptococcus mutans*, *E. coli*, and *S. aureus*. Tested fungal are displayed in Figure 2. *C. albicans* was the lowest (1.3845 OD), whereas *A. niger* was (2.407 OD). **Salim *et al.* [35]** stated that the ethanol extract of *costus* rhizome demonstrated antifungal activity against three investigated fungal species (*Aspergillus fumigates, Fusarium species, and Penicillium species*). Triterpenoids, flavonoids, steroids, and sesquiterpene lactones, which are abundant in *S. costus*, might possess a number of biological properties, such as antimicrobial properties **[36]**.

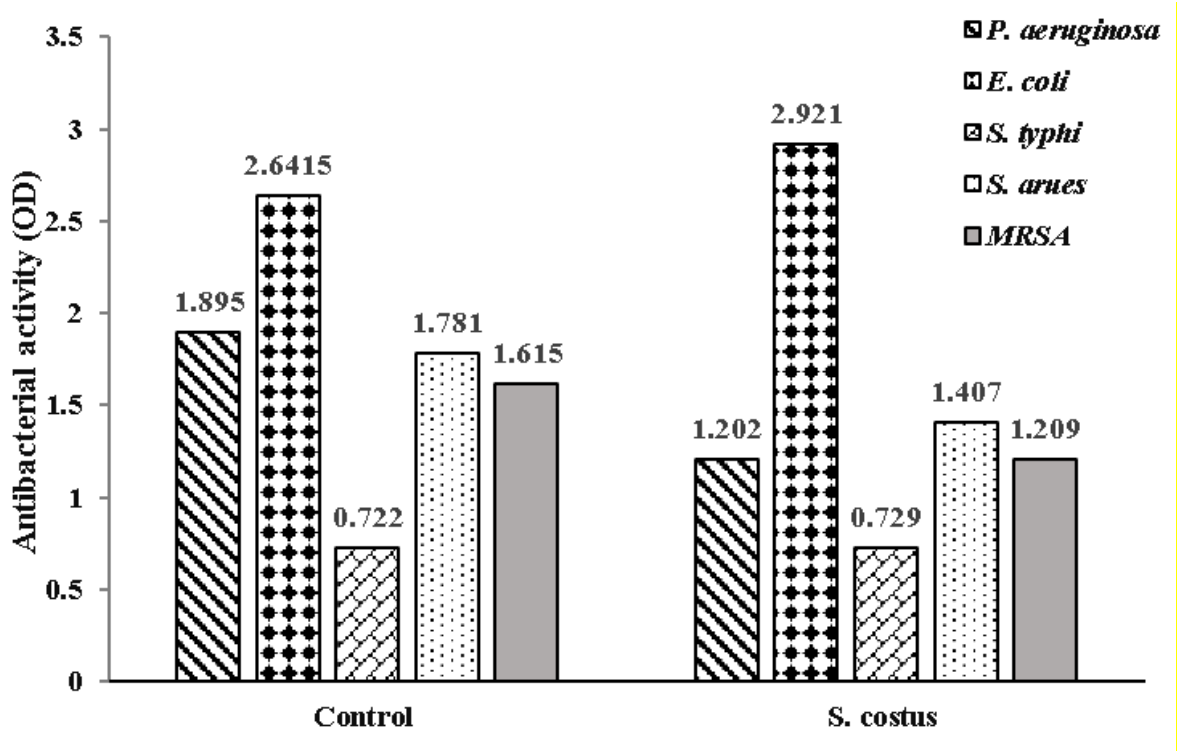

Figure 1: Antibacterial activity of *S. costus* roots

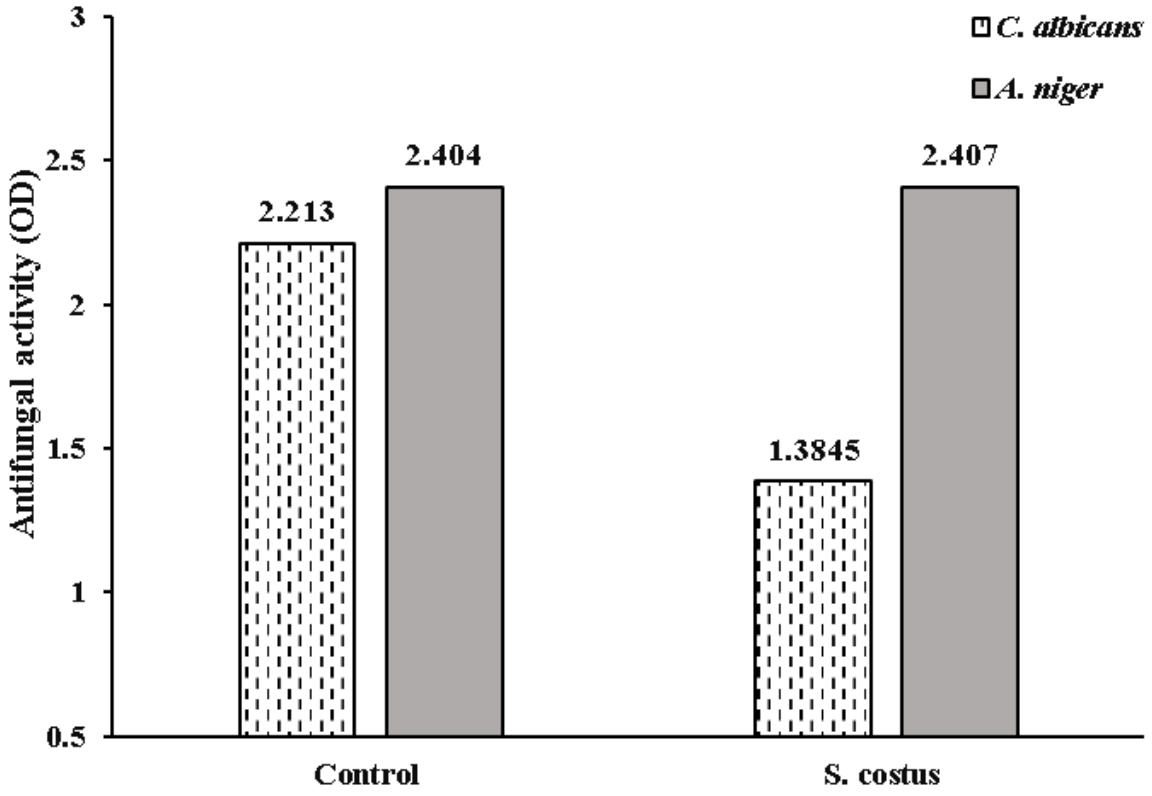

Figure 2: Antifungal activity of *S. costus* roots

Table 3: Minimum inhibitory concentration (MIC) of *S. costus* roots

Test microorganism	MIC (µg /ml)
P. aeruginosa	250
E. coli	62.5
S. typhi	250
S. arues	250
MERSA	62.5
C. albicans	100
A. niger	200

3.4. Cytotoxic activity of *S. costus* roots

The cytotoxicity of *Saussurea costus* root on normal cells was assessed by evaluating its effects across a range of concentrations, (0 to 500 µg/ml). The results in Table 4 showed that *S. costus* was safe. The results of cytotoxicity screening of *S. costus* roots against the Vero cells expressed an IC_{50} value of 70.50±3.17 µg/ml. The absence of cytotoxicity may be attributed to the phytochemicals compounds in *S. costus*, which could have protective effects against oxidative stress and inflammation while preventing apoptosis in normal cells **[37]**. An acute oral toxicity study was conducted using an aqueous extract of *S. costus* at doses up to 2000 mg/kg body weight, in accordance with **Saleem *et al.* [38].** In another study, **Khalid *et al.* [39]** stated that doses of *S. lappa* root ethanolic extract up to 5000 mg/kg did not induce mortality in animals throughout the experimental period. The findings indicated no evidence of toxicity or adverse effects on hematological, biochemical parameters, or histopathological examination of the liver and kidneys.

Table 4: cytotoxic activity for *S. costus*

Sample conc. (µg/ml)	Viability %
500	5.46
250	13.58
125	30.67
62.5	52.84
31.25	71.32
15.6	88.19
0	100
IC_{50}	70.50±3.17 µg/ml

3.5. Effect of *S. costus* roots on Acetylcholine esterase enzyme (AChE)

Alzheimer's is a disease among the major global public health priorities, and recently, its occurrence has been increasing at an alarming rate. AChE breaks down acetylcholine, a neurotransmitter essential for memory and learning. In Alzheimer disease (AD), the cholinergic hypothesis suggests that the loss of acetylcholine contributes to cognitive decline **Zanati *et al* [40]**. Therefore, the primary target of therapeutic medications approved for (AD) is based on the "cholinergic hypothesis," which focuses on the inhibition of acetylcholinesterase (AChE) **Mangialasche *et al.* [41]**.

Table 5 represents the inhibition % of the Acetylcholine esterase enzyme (AChE), which may be considered one of the factors associated with Alzheimer's disease in humans. The results showed that the inhibition (%) had increased with increasing concentration of *S. costus* roots used until 500 µg/ml. Thus, it was 72.605 % with IC_{50} (155.3±10.28 µg/ml) compared to the control (Donepezil standard was 0.313± 0.0023 µg/ml**)**.

Remarkable research by **Narimane *et al.* [42]** evaluated the encapsulated and nano-encapsulated forms of *Saussurea lappa* (synonymous with *S. costus*) essential oil for their acetylcholinesterase (AChE) inhibitory activity. The findings indicated that both encapsulated and nano-capsulated forms showed inhibition of AChE in a dose-dependent manner, but the nano-capsulated form enhanced efficacy which suggests that *S. costus* essential oil possesses anti-Alzheimer properties, potentially due to its sesquiterpene lactones, like costunolide and dehydrocostus lactone, which may interact with enzyme activity sites. Also, other studies by **Ibrahim *et al.* [43] Hajimehdipoor *et al.* [44]**, and **Hegazy *et al.* [45]** revealed that the methanolic extract of *S. costus* roots contains sesquiterpene lactones that have been identified as cholinesterase inhibitors. As previously mentioned, catechin was recognized as one of the principal phenolic chemicals in *S. costus* roots. **Özduran *et al.* (46)** stated the polyphenolic compounds known as catechins are important because of their anti-inflammatory, anti-apoptotic, and antioxidant qualities, which help prevent and cure neurodegenerative disorders. Catechins' medicinal properties have been thoroughly investigated in both human and animal models.

Table 5: Inhibition of Acetylcholine esterase enzyme (AChE) activity by *S. costus*

Conc. (µg/mL)	**31.25**	**62.5**	**125**	**250**	**500**
% inhibition	8.079	27.622	43.063	66.579	72.605
IC_{50}					
***S. costus* extract**	155.3±10.28 (µg/ml)				
Standard donepezil	0.313± 0.0023 (**µ**g/mL)				

3.6. Crackers analysis

3.6.1. Chemical composition of substituted crackers with *S. costus* roots

The chemical composition of crackers substituted with *S. costus* roots powder at ranges from 1 to 4% is shown in **Table 6**. The moisture content of the crackers ranged from 4.37 to 4.16 g/100g, showing a significant reduction compared to the control (5.36%). Moisture plays a crucial role in determining the quality, acceptability, and shelf life of baked goods, as it influences texture, microbial stability, and overall product freshness **[47].**

For protein content, the control sample displayed higher levels compared to the substituted crackers, with significant differences observed among the treatments. Conversely, the fat and fiber content of the control crackers was lower than that of the crackers substituted with *S. costus* roots. Additionally, crackers substituted with *S. costus* roots demonstrated a higher ash content compared to the control sample.

Interestingly, the available carbohydrate content remained consistent between the control crackers and those substituted with *S. costus* powder. In terms of energy, the control crackers provided 403.99 kcal/100 g, while the energy value of crackers substituted with *S. costus* roots increased slightly to 406.54 and 406.10

kcal/100 g for 1% and 2% samples espectively. Meanwhile, energy value was 405.35 and 405.44 kcal/100 g for the 3 and 4% samples, respectively. Whereas **Maisuthisakul *et al*. [48],** found that rice crackers enriched with *Cratoxylum formosum* extract had low fat (1.8 g/100 g) and high total carbohydrate content (79.3 g/100 g)donmenstrate low fat1.8 g/100 g, high total carbohydates 79.3 g/100 g.

Table 6: Chemical composition of substituted crackers with *S. costus* roots powder on dry weight (g/100g)

Sample	Moisture	Protein	Fat	Fiber	Ash	*Available Carbohydrates	Energy Kcal/100g
Control	5.36^{a} ±0.03	9.45^{a} ±0.01	10.06^{e} ±0.01	2.38^{c}±0.05	1.57^{e}±0.02	71.18^{b}±0.07	403.99^{b}±0.03
1%	4.37^{b}±0.01	9.42^{a}±0.02	10.15^{d}±0.05	2.40^{b}±0.02	2.02^{d}±0.05	71.64^{a}±0.01	406.54^{a}±0.05
2%	4.30^{c}±0.01	9.38^{b}±0.01	10.19^{c}±0.05	2.45^{ab}±0.01	2.10^{c}±0.11	71.58^{a}±0.13	406.10^{a}±0.10
3%	4.22^{d}±0.02	9.32^{c}±0.01	10.26^{b}±0.01	2.48^{a}±0.01	2.52^{b}±0.01	71.20^{b}±0.02	405.35^{ab}±0.06
4%	4.16^{e}±0.05	9.22^{d}±0.05	10.32^{a}±0.05	2.50^{a}±0.01	2.61^{a}±0.01	71.19^{b}±0.03	405.44^{ab}±0.04

Values in the same column with different letters are significantly different (P ≤0.05).

* Available carbohydrate=100 - (Moisture+ Protein+Fat+ Fiber+Ash)

3.6.2. Sensory evaluation of crackers with *S. costus* roots

The acceptance of a new product is primarily influenced by its organoleptic properties. Statistical analysis of organoleptic evaluation is displayed in **Table 7.** The given scores revealed that panelists approved the samples except for those containing a 4% concentration of *S.costus* root powder, which were noted to have a bitter aftertaste. From the data, it could be concluded that the organoleptic properties decrease with the increase in the percentage of S. *costus* root powder. Color scores significantly reduced from 8.90 to 7.20 ($p<0.05$) with increasing *S. costus* concentration, demonstrating a negative effect on product appearance.

Taste is an important property and is a crucial part of sensory evaluation. The results in **Table 7** indicate that the panelists found the newly designed crackers taste generally satisfactory, with ratings ranging from 9.45 to 7.5.

One of the key characteristics of crackers is their texture; the most popular types are crispy and crackly, according to **Saeleaw and Schleining [49],** which is characterized by sound emission at low fracture forces. The ratings for the crispiness of the crackers varied slightly with different formulations, ranging from 7.80 to 9.65. These results indicate that the crackers generally exhibited favorable crispiness, with minor variations depending on the specific formula used.

Rakesh and Datta [50] defined puffing as a rapidly cooking process at high temperatures that evaporates moisture within starch, expanding the product into a porous structure. The Puffiness scores for the crackers varied between 6.5 and 9.0, indicating moderate to high acceptability with some formulation-dependent variations.

Table 7: Sensory evaluation of crackers with *S. costus* roots powder

Items	Color	Puffing	Taste	Crispiness	Texture	Overall Score
Control	8.90^{a}± 0.06	9.00^{a}± 0.03	9.45^{a}± 0.01	9.65^{a}± 0.05	9.00^{a}± 0.04	8.63^{b} ± 0.04
1%	8.80^{a}± 0.05	8.95^{ab} ± 0.04	9.30^{ab}± 0.03	9.23^{b}± 0.07	8.90^{ab}± 0.05	8.00^{c} ± 0.07
2%	8.15^{b}± 0.04	8.80^{b}± 0.04	9.10^{b}± 0.03	9.00^{c}± 0.07	8.85^{b}± 0.05	8.60^{b} ± 0.05
3%	7.98^{c}± 0.07	8.70^{c}± 0.06	9.00^{b}± 0.06	9.05^{c}± 0.03	8.80^{b}± 0.05	8.80^{a} ± 0.02
4%	7.20^{d}± 0.08	6.50^{d}± 0.09	7.50^{c}± 0.03	7.80^{d}± 0.05	6.50^{c}± 0.02	6.14^{d} ± 0.06

Values in the same column with different letters are significantly different (P ≤0.05).

3.6.3. Phytochemical activity of substituted crackers with *S. costus* roots

The DPPH approach relies on a sample's capacity to donate hydrogen in order to scavenge free radicals. **Table 8** showed an increase in the antioxidant activity of crackers, particularly those substituted with *S. costus* roots, compared to the control sample during the storage period. After three months of storage at room temperature, the DPPH inhibition percentages for 3% and 4% were 68.65 and 72.90 respectively. Consequently, control crackers made of wheat flour only had the lowest level of antioxidant activity. Replacing a portion of the wheat flour with *S. costus* powder in the cracker recipe significantly enhanced the antioxidant properties of the final product.

Total phenol and flavonoid contents are shown in **Table 8**. The results showed that total phenol content (TPC) was significantly higher than total flavonoid content (TFC) at all substitution levels. Both TPC and TFC increased as the percentage of *S. costus* root powder in the crackers increased. After three months of storage, crackers containing 4% *S. costus* powder had the highest TPC (186.33 mg GAE/100g) and TFC 74.70 mg

QC/100g). These reductions may be attributed to prolonged storage, often leading to oxidative stress or interaction with polyphenol oxidases, which can reduce TPC and TFC levels **[50]** also,these results are parallel with **Ibadullah *et al.* [51].** Since medicinal plants are known to be rich sources of polyphenols, which are commonly used to enhance the functional properties of various food items, **Qadir *et al.* [52].** In a previous study by **Starowicz and Zieliński [53]**, during storage for 18 months, cakes mixed with cloves, nutmeg, cinnamon, vanilla, allspice, and a commercial blend of spices, the antioxidant capacity was decreased, which highly correlated with the contents of total phenols, flavonoids.

Crackers prepared with wheat flour with varying percentages (1, 2, 3, and 4%) of *S. costus* powder increased the phenolic compounds in the crackers. Consequently, they improved their antioxidant capacity without compromising their acceptability or sensory quality.

Table 8: Phytochemical activity of substituted crackers with *S. costus* roots

Formulas	DPPH %	TPC mg GAE/100g	TFC mg QC/100g
Zero time			
Control	$41.64^{e}\pm2.33$	$110.90^{e}\pm0.78$	$14.57^{e}\pm0.06$
1%	$56.00^{d}\pm0.22$	$140.79^{d}\pm0.7$	$34.72^{d}\pm0.44$
2%	$64.89^{c}\pm0.79$	$161.87^{c}\pm0.81$	$42.49^{c}\pm0.45$
3%	$72.49^{b}\pm1.28$	$173.66^{b}\pm1.52$	$72.73^{b}\pm0.63$
4%	$75.87^{a}\pm0.61$	$192.33^{a}\pm5.5$	$79.40^{a}\pm0.39$
After 3 months of storage			
Control	$24.05^{e}\pm1.39$	$87.86^{e}\pm1.02$	$9.43^{e}\pm0.22$
1%	$49.95^{d}\pm0.35$	$119.70^{d}\pm0.617$	$28.79^{d}\pm0.2$
2%	$59.00^{c}\pm1.35$	$155.92^{c}\pm0.88$	$37.85^{c}\pm0.16$
3%	$68.65^{b}\pm0.25$	$162.96^{b}\pm2.06$	$67.06^{b}\pm0.5$
4%	$72.90^{a}\pm1.67$	$186.33^{a}\pm3.21$	$74.70^{a}\pm0.6$

Values in the same column with different letters are significantly different ($P \leq 0.05$) at each storage period. TPC: total phenolic compound, TFC: total flavonoid compounds.

3.6.4. Microbial assay during the storage period of substituted crackers with S. costus roots powder

Numerous antibacterial properties of *S. costus* make it a viable substitute for artificial preservatives that endanger human health. **[3]**. The results in Table *9* showed that there was no detection of total bacteria count, total yeast, and mold at zero time in the control and substituted crackers. It was demonstrated that control crackers increased by 4.80 log cfu.g^{-1} and 4.77 log cfu.g^{-1}, respectively, after three-month storage periods. Meanwhile, the total bacterial count for yeast and mold was low in crackers at different concentrations of *S. costus*. The microbial count has decreased with the increase in the concentration of *S. costus*. It was noticed that the sample with 4% has no detection of microbial growth. The lowest microbial count inhibition was recorded in the sample with 1% 3.30 log cfu.g^{-1} and 3.04 log cfu.g^{-1} for total microbial count, yeast, and mold, respectively. These results are due to the higher percentage of various phenolic and flavonoid compounds present in *S. costus.*

The activity of *S.costus* roots against pathogenic fungi indicated that they have moderate antifungal activity against *Rhizopus stolonifer* and *A. tamari* **Srinivasan *et al* [54]**. The phytochemical of *S. costus* is responsible for its antibacterial qualities. **Idriss *et al.* [55]**. These compounds exhibit antibacterial qualities supporting the use of *S. costus* as a natural antimicrobial agent. Furthermore, the antifungal activity of *S. costus* against Candida species has been assessed **Soliman *et al.* [56]**. These findings highlight the potential of *S. costus* root extracts as effective medicinal agents with both antibacterial and antifungal applications.

Table 9: Microbial assay during the storage period of substituted crackers with S. costus roots powder (log cfu.g^{-1})

Analysis	Total bacteria count		Yeast & Mold	
Storage / Formulas	Zero time	3 months	Zero time	3 months
Control	ND	4.80	ND	4.77
1%	ND	3.30	ND	3.04
2%	ND	3.14	ND	2.84
3%	ND	3.00	ND	2.69
4%	ND	2.60	ND	ND

ND = Not detected

4. Conclusion

The current study demonstrates that *Saussurea costus* (*S. costus*) represents a promising source of bioactive compounds, exhibiting potent biological activities, including antimicrobial, antifungal, and antioxidant properties. High-performance liquid chromatography (HPLC) analysis identified flavonoids and phenolic compounds as key constituents, which correlated with significant DPPH free radical scavenging activity. Furthermore, it was discovered that *S. costus* inhibits acetylcholinesterase (AChE), which has become the primary treatment target for Alzheimer's disease. S. costus shows applicability as a functional ingredient with nutritional, antimicrobial, and potential neuroprotective benefits. Its incorporation into crackers at a low concentration (up to 3% flour replacement) enhances nutritional value. It extends shelf-life without compromising sensory acceptability, as higher substitution levels were found to be sensorially unfavorable in consumer evaluations. These findings position *S. costus* as a viable candidate for nutraceutical and food preservation applications, highlighting its potential for incorporation into functional food formulations.

5. References

[1]. M.M. Deabes, A.K. Allayeh, M.M. Seif, A.-H.M. Rasmey, K.M. Naguib, Antiviral, antifungal, and antibacterial potential activities of ephedra sinica in vitro, Jordan Journal of Biological Sciences 13(3) (2020).

[2]. M. Deabes, W. Aboulthana, E.E.-D.M.A. Marzouk, M.I. Mohamed, K.A. Ahmed, Evaluation of hepato- and neuroprotective effect of chemical constituents in saussurea costus extract against the toxicity induced by chloropyrifos ethyl in rats, Egyptian Journal of Chemistry 64(2) (2021) 631-647.

[3]. G.A. El-Chaghaby, F.S. Mohammed, S. Rashad, I. Uysal, O. Koçer, Ö. Lekesiz, M. Doğan, A.E. Şabik, M. Sevindik, Genus hypericum: General properties, chemical contents and biological activities, Egyptian Journal of Botany 64(1) (2024) 1-26.

[4]. M.E. Abdel-Alim, M.S. Serag, H.R. Moussa, M.A. Elgendy, M.T. Mohesien, N.S. Salim, Phytochemical screening and antioxidant potential of lotus corniculatusand amaranthus viridis, Egyptian Journal of Botany 63(2) (2023) 665-681.

[5]. B. Wani, F. Mohammad, A. Khan, R. Bodha, F. Mohiddin, A. Hamid, Some herbs mentioned in the holy quran and ahadith and their medicinal importance in contemporary times, J Pharm Res 11 (2011) 3888-3891.

[6]. T. Julianti, Y. Hata, S. Zimmermann, M. Kaiser, M. Hamburger, M. Adams, Antitrypanosomal sesquiterpene lactones from saussurea costus, Fitoterapia 82(7) (2011) 955-959.

[7]. H.A. El Gizawy, A.E. El-Haddad, A.M. Saadeldeen, S.A. Boshra, Tentatively identified (uplc/t-tof–ms/ms) compounds in the extract of saussurea costus roots exhibit in vivo hepatoprotection via modulation of hnf-1α, sirtuin-1, c/ebpα, mirna-34a and mirna-223, Molecules 27(9) (2022) 2802.

[8]. E.A. Abdelghffar, N.M. Mostafa, H.A. El-Nashar, O.A. Eldahshan, A.N.B. Singab, Chilean pepper (schinus polygamus) ameliorates the adverse effects of hyperglycaemia/dyslipidaemia in high fat diet/streptozotocin-induced type 2 diabetic rat model, Industrial Crops and Products 183 (2022) 114953.

[9]. H. Fan, F. Liu, S.A. Bligh, S. Shi, S. Wang, Structure of a homofructosan from saussurea costus and anti-complementary activity of its sulfated derivatives, Carbohydrate polymers 105 (2014) 152-160.

[10]. M. Ashry, D. El-Sahra, K. abdel-wahhab, M. Abdelsalam, H. Mourad, A. El-Bitar, H. Gomaa, Saussurea costus extract has anti-inflammatory, antioxidant, and hormonal effects against testicular toxicity induced by oxaliplatin in male albino rats, Iranian Journal of Toxicology 16 (2022) 83-90.

[11]. A. Robinson, T.V. Kumar, E. Sreedhar, V. Naidu, S.R. Krishna, K.S. Babu, P. Srinivas, J.M. Rao, A new sesquiterpene lactone from the roots of saussurea lappa: Structure–anticancer activity study, Bioorganic & medicinal chemistry letters 18(14) (2008) 4015-4017.

[12]. S. Hasson, M. Al-Balushi, K. Alharthy, J. al-busaidi, M. Aldaihani, M. Othman, E. Said, O. Habbal, T.A. Sallam, A. Aljabri, M. Idris, A. Wadieh, Evaluation of anti–resistant activity of auklandia(saussurea lappa) root against some human pathogens, Asian Pacific journal of tropical biomedicine 3 (2013) 557-62.

[13]. A. Al Ghasham, M. Al Muzaini, K.A. Qureshi, G.O. Elhassan, R.A. Khan, S.A. Farhana, S. Hashmi, E. El-Agamy, W.E. Abdallah, Phytochemical screening, antioxidant and antimicrobial activities of methanolic extract of ziziphus mauritiana lam. Leaves collected from unaizah, saudi arabia, International Journal of Pharmaceutical Research & Allied Sciences 6(3) (2017).

[14]. H.F. Aly, E. Younis, A. Gaafar, S.G.E. SHAMS, K.A. Ahmed, H. Abu Hashish, Z. Salama, The efficacy of egyptian clementine oil identified by gc/ms analysis on alzheimer's disease –induced rats, Egyptian Journal of Chemistry 65(3) (2022) 465-477.

[15]. M. Mathew, S. Subramanian, In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders, PLoS One 9(1) (2014) e86804.

[16]. AOAC international, 21st edition, official methods of analysis association of official analytical chemists, Association of Official Analytical Chemists,, Washington DC., 2019.

[17]. E.H. Mansour, A.H. Khalil, Characteristics of low-fat beefburger as influenced by various types of wheat fibers, Food Research International 30(3-4) (1997) 199-205.

[18]. R. Fu, Y. Zhang, Y. Guo, F. Liu, F. Chen, Determination of phenolic contents and antioxidant activities of extracts of jatropha curcas l. Seed shell, a by-product, a new source of natural antioxidant, Industrial Crops and Products 58 (2014) 265-270.

[19]. S.R. Kanatt, K. Arjun, A. Sharma, Antioxidant and antimicrobial activity of legume hulls, Food Research International 44(10) (2011) 3182-3187.
[20]. B. de Ancos, S. Sgroppo, L. Plaza, M.P. Cano, Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment, Journal of the Science of Food and Agriculture 82(8) (2002) 790-796.
[21]. K.-H. Kim, R. Tsao, R. Yang, S.W. Cui, Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions, Food Chemistry 95(3) (2006) 466-473.
[22]. M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review, Journal of pharmaceutical analysis 6(2) (2016) 71-79.
[23]. Y. Kia, H. Osman, S.K. Raju, A. Basiri, V. Murugaiyah, Ionic liquid mediated synthesis of mono- and bis-spirooxindole- hexahydropyrrolidines as cholinesterase inhibitors and their molecular docking studies, Bioorganic & medicinal chemistry 22 (2014).
[24]. G. Ellman, K.D. Courtney, V. Andres, R. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochemical Pharmacology 7 (1961) 88-95.
[25]. J.J. Han, J.A. Janz, M. Gerlat, Development of gluten-free cracker snacks using pulse flours and fractions, Food Research International 43(2) (2010) 627-633
[26]. H. Stone, R.N. Bleibaum, H.A. Thomas, Sensory evaluation practices, Academic press2020.
[27] E.A. Akl, M.A. Younos, A comparative study on bioactive compounds and biological activities of ethanolic extracts of saussurea costus and withania somnifera, Egyptian Journal of Botany 64(3) (2024) 809-823.
[28]. S.F.M. Allam, W.S.M. Amin, Chemical composition, bioactive compounds and antioxidant activity of the aqueous and ethanolic extracts from saussurea lappa roots, The Seybold Report 17(106) (2022) 1760-1774.
[29]. M. Premalatha, S. Lakshmi, A study on the antioxidant and antimicrobial activities in the ethyl acetate extract of saussurea lappa, International Journal of Current Research 12(07) (2020) 12662-12667.
[30]. A. Hashimi, A. Siddiqui, Y. Ahmed, M.B. Siraj, R. Khatoon, Quality control and phytochemical validation of saussurea lappa (costus/qust), International Journal of Green Pharmacy (IJGP) 14(1) (2020) 1-6.
[31]. Z.A. Al-Zayadi, H.K. Shanan, K.A. Al Salihi, Extraction and evaluation of active ingredients of saussurea costus roots and determination of its antibacterial activity, IOP conference series: earth and environmental science, IOP Publishing, 2023, p. 012058.
[32]. M. Ashry, Protective effect of costus (saussurea costus) ethanolic extract on oxaloplatin®-induced histological changes and hemato-cardiotoxicity in adult male albino rats, Egyptian Academic Journal of Biological Sciences, D. Histology & Histochemistry 11(2) (2019) 69-85.
[33]. N. Mammate, F. El Oumari, H. Imtara, S. Belchkar, G. Benjelloun Touimi, A.-Z. Mohammed, H. Rudayni, A. Qurtam, M. Aleissa, F. Nasr, O. Noman, T. Houssaini, Anti-struvite, antimicrobial, and anti-inflammatory activities of aqueous and ethanolic extracts of saussurea costus (falc) lipsch asteraceae, Molecules (2023).
[34]. G. Gutiérrez-Venegas, J.A. Gómez-Mora, M.A. Meraz-Rodríguez, M.A. Flores-Sánchez, L.F. Ortiz-Miranda, Effect of flavonoids on antimicrobial activity of microorganisms present in dental plaque, Heliyon 5(12) (2019).
[35]. F.A. Salim, H.A. Diab, A.K. Hmedan, H.N.E. Dhidah, R.E. Baayo, S. Hussain, A study of antibacterial, antifungal activities of ethanolic and aqueous extracts of costus speciosus, The Pharmaceutical and Chemical Journal 6(1) (2019) 11-18.
[36]. S.I. Abdelwahab, M.M.E. Taha, H.A. Alhazmi, W. Ahsan, Z. ur Rehman, M. Al Bratty, H. Makeen, Phytochemical profiling of costus (saussurea lappa clarke) root essential oil, and its antimicrobial and toxicological effects, Tropical Journal of Pharmaceutical Research 18(10) (2019) 2155-2160.
[37]. S.-M. Moon, S.J. Yun, J.-K. Kook, H.-J. Kim, M.S. Choi, B.R. Park, S.-G. Kim, B.-O. Kim, S.-Y. Lee, H. Ahn, Anticancer activity of saussurea lappa extract by apoptotic pathway in kb human oral cancer cells, Pharmaceutical biology 51(11) (2013) 1372-1377.
[38]. T.M. Saleem, N. Lokanath, A. Prasanthi, M. Madhavi, G. Mallika, M. Vishnu, Aqueous extract of saussurea lappa root ameliorate oxidative myocardial injury induced by isoproterenol in rats, Journal of advanced pharmaceutical technology & research 4(2) (2013) 94-100.
[39]. A.S. Khalid, I. Momodu, M.U. Iduh, B. Sirajo, H.S. Khalid, U. Abubakar, A. Inioyola, H.I. Wasagu, Toxicity assessment of ethanol extract of saussurea lappa (costus) root in male wistar rats, African Journal of Biology and Medical Research 7(4) (2024).
[40]. M. Znati, A. Zardi-Bergaoui, M. Daami-Remadi, H. Ben Jannet, Semi-synthesis, antibacterial, anticholinesterase activities, and drug likeness properties of new analogues of coumarins isolated from ferula lutea (poir.) maire, Chem. Afric. 3 (2020) 635-645.
[41]. F. Mangialasche, A. Solomon, B. Winblad, P. Mecocci, M. Kivipelto, Alzheimer's disease: Clinical trials and drug development, The Lancet Neurology 9(7) (2010) 702-716.
[42]. N. Lammari, T. Demautis, O. Louaer, A.H. Meniai, H. Casabianca, C. Bensouici, G. Devouassoux, H. Fessi, A. Bentaher, A. Elaissari, Nanocapsules containing saussurea lappa essential oil: Formulation, characterization, antidiabetic, anti-cholinesterase and anti-inflammatory potentials, International Journal of Pharmaceutics 593 (2021) 120138.
[43]. M. Ibrahim, T. Farooq, N. Hussain, A. Hussain, T. Gulzar, I. Hussain, M.S.H. Akash, F.S. Rehmani, Acetyl and butyryl cholinesterase inhibitory sesquiterpene lactones from amberboa ramosa, Chem. Cent. J. 7 (2013) 1-5.
[44]. H. Hajimehdipoor, M. Mosaddegh, F. Naghibi, A. Haeri, M. Hamzeloo-Moghadam, Natural sesquiterpen lactones as acetylcholinesterase inhibitors, Anais da Academia Brasileira de Ciências 86 (2014) 801-806.
[45]. M.-E.F. Hegazy, A.Y. Ibrahim, T.A. Mohamed, A.A. Shahat, A.M. El Halawany, N.S. Abdel-Azim, M.S. Alsaid, P.W. Paré, Sesquiterpene lactones from cynara cornigera: Acetyl cholinesterase inhibition and in silico ligand docking, Planta medica 82(01/02) (2016) 138-146.

[46]. A.O. Adebayo-Oyetoro, O.O. Ogundipe, K.N. Adeeko, Quality assessment and consumer acceptability of bread from wheat and fermented banana flour, Food Science & Nutrition 4(3) (2016) 364-369.

[47] Özduran, G., Becer, E. and Vatansever, H. S. (2021). The Role and Mechanisms of Action of Catechins in Neurodegenerative Diseases. Journal of the American College of Nutrition Journal of the American College of Nutrition, DOI: 10.1080/07315724.2021.1981487

[48] Maisuthisakul, P., Gordon, M. H., Pongsawatmanit, R., & Suttajit, M. (2007). Enhancing the oxidative stability of rice crackers by addition of the ethanolic extract of phytochemicals from Cratoxylum formosum Dyer. Asia Pacific journal of clinical nutri

[49]. M. Saeleaw, G. Schleining, Effect of frying parameters on crispiness and sound emission of cassava crackers, Journal of Food Engineering 103(3) (2011) 229-236.

[50]. V. Rakesh, A. Datta, Microwave puffing: Mathematical modeling and optimization, Procedia Food Science 1 (2011) 762-769.

[51]. W.Z.W. Ibadullah, A.A. Idris, R. Shukri, N.A. Mustapha, N. Saari, N.H.Z. Abedin, stability of fried fish crackers as influenced by packaging material and storage temperatures, Current Research in Nutrition and Food Science Journal 7(2) (2019) 369-381.

[52]. M.B. Qadir, F. Mateen, A.G. Al-Sehemi, Enhanced charge transport characteristics in zinc oxide nanofibers via mg2+ doping for electron transport layer in perovskite solar cells and antibacterial textiles, (2022).

[53]. M. Starowicz, H. Zieliński, Changes in the antioxidant capacity and polyphenols content of rye-buckwheat cakes fortified with spices during their long-term storage: English, Ital J Food Sci 31(2) (2019).

[54]. G. Srinivasan, K. Vijayan, P. Sharanappa, K. Jagadeesh, Identification of chemical compounds in the essential oil from costus pictus d. Don plant parts antimicrobial studies isolation and quantification of diosgenin from its root, Journal of Chemical and Pharmaceutical Research 8(7) (2016) 594-604.

[55]. H. Idriss, B. Siddig, P. González-Maldonado, H. Elkhair, A.I. Alakhras, E.M. Abdallah, A.O. Elzupir, P.H. Sotelo, Inhibitory activity of saussurea costus extract against bacteria, candida, herpes, and sars-cov-2, Plants 12(3) (2023) 460.

[56]. M.F. Soliman, Y.M. Shetaia, A.A. Tayel, A.M. Munshi, F.A. Alatawi, M.A. Alsieni, M.A. Al-Saman, Exploring the antifungal activity and action of saussurea costus root extracts against candida albicans and non-albicans species, Antibiotics 11(3) (2022) 327.