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PHYTOCHEMICAL SCREENING THROUGH GCMS AND PHARMACOLOGICAL EVALUATION OF *ALLIUM ASCALONICUM*

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Abstract

Due to their deeply ingrained beliefs, many communities throughout the world include *Allium ascalonicum (AAS)* in their diets. Earlier reports on AAS extracts proves its anticancer and antibacterial efficiency. Current research focused the Phytochemical Screening through GCMS and Pharmacological Evaluation of Alliumas Ascalonicum. The AAS plant was collected from to Waziristan and Bannu, KP Pakistan, in September 2019 and confirmed by Faculty of Pharmacy, Gomal University DI Khan, Pak. The aerial components of the AAS were collected and sliced into small parts and extracts in various solvents along with crude drug were prepared. Various compounds were identified using GCMS screening and 35 compounds were screened. The activities, antioxidant assay, urease assay, and anticholinesterase assay were carried out with crude (Cr), fixed oil, ethyl acetate (EtAc), chloroform (Chf), hexane (Hex), and aqueous (Aq) extracts. Significant results were obtained with EtAc, and fixed oil for these activities. The screened compounds and marked pharmacological activities make AAS as one of the most significant medicinal dietary plants to be further evaluated for clinical utilization. The current research gives an outlook for the future evaluation of AAS and AAS derived compounds including its fixed oils against various pharmacological uses.

Index Terms: *Alliumas Calonicum*, Anti-Cholinesterase Activity, Antioxidant Activity, Gcms Analysis, Phytochemical Screening, Urease Activity.

1. INTRODUCTION

The nature has gifted with various natural resources but to which extent is not accessible. The plants of the natural resources that are essential to meeting life's fundamental requirements. Medicinal plants are used for various purposes throughout the world and approximately 2.5 billion dollars ($USD) have been made through the sales of different natural resources, as per trustworthy sources. Including over 0.4 million species that make up the kingdom plantae serve as a source of naturally occurring bioactive chemicals [1]. Different therapeutic components of herbal medicine have been processed into different forms and afterwards given to both cattle and people via numerous suitable routes [2].

Allium ascalonicum (AAS) is one of the very significant species of Liliaceae family [3, 4]. AAS is amongst the primogenital cultured vegetables and have been used in spices, vegetables, ornamentals, or as medicines for the treatment of many manifestations [5]. The earliest Charaka Samhita (Hindu scripture) report that these plants possess some

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medicinal value [6]. There are 750 species and 30 genera in the Allium genus, which is extensively scattered around the world [7]. It is a herb that is grown in Asia that has a slight onion-like scent. The shallot is more popular among Asians than the regular onion [9]. Since ancient times, people have employed their bulbs, leaves, and inflorescences to prepare a variety of foods, soups, and traditional remedies [10], [11]. Typically, shallots generate groups of 2 to 20 bulb pieces, each measuring between 30 and 40 mm in diameter [12]. Even if seed production is possible, some shallot genotypes in tropical locations seldom blossom, and shallot genotypes are often reproduced by cloning. [13]. from this family, many chemicals have been isolated [3].

Over the decades, It was discovered that certain Allium species have therapeutic properties and are used traditionally as painkillers, anti-inflammatory treatments, stomach ailments, anthelmintics, and analgesics [14], anti-fungal, hypoglycemic agent [15], anti-oxidative [16], beneficial hematological influences [4], antiviral [17], anti-Helicobacter pylori potential [18, 19] peroxynitrite-scavenging capacity [20], hypocholesterolemic [21], antiparasitic activities [20], and in vitro anti-bacterial activities of shallot [22].

There are several species of the genus Allium that may be spotted all over the world. The AAS is inhabitant to the areas including Waziristan and Bannu, KP Pakistan [22] this species is intriguingly tasty and has been used for feed and food, although it is entirely unexplored. The present study effort has been designed to examine different pharmacological characteristics and the toxicity profile of AAS samples based on literature review and ethnomedical applications. In the current research study, fixed oil of the AAS was collected by converting the aerial parts of the AAS into small pieces and subsequently. GS-MS analysis was performed for the complete phytochemical screening of AAS crude extract. The current research also focused on the evaluation of AAS and AAS derived compounds including its fixed oils against various pharmacological uses.

2. MATERIALS AND METHODS

2.1 Materials

2.2 Collection, Identification, and Extraction

The AAS plant was harvested during the blossoming period and dried in the shade to prevent the formation of fungus. The plant was macerated and filtered after being pulverised. The resulting filtrate was evaporated at lower pressure using a rotary evaporator. The crude extract was fractionated utilizing a sequential solvent-solvent extraction method in a separating funnel. Also, every crude sample's percentage yield was computed.

2.3 GC-MS Analysis

Plant samples including solvent fractions, and crude sample was evaluated through GC-MS analysis [23]. A standard method was used to run GC-MS instrument. Identification was based on the molecular structure, molecular mass, and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National

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Institute Standard and Technology (NIST) library.

2.4 Antioxidant Activities

To evaluate the antioxidant capability of AAS extract, we performed DPPH assay. The assay was performed using spectrophotometer for measuring the ability of the sample to reduce α, α-diphenyl-β-picrylhydrazyl (DPPH; C18H12N5O6, M = 394.33). By adding a hydrogen atom from antioxidants to the corresponding hydrazine, the odd electron of the nitrogen atom in DPPH is reduced. The UV absorption spectra was measure at optimum wavelength 517 nm. DPPH is standard recommendation for antioxidant activity, owing to its sensitivity to spectrophotometry, light, and pH [24].

We also performed the procedure to measure H2O2 contents in the sample of AAS extract. Spectrophotometric measurements were carried out at 415nm for titanium-performed complex to determine the H2O2 contents. A typical protocol was used where fresh frozen sample of AAS (200 mg) was homogenized in 2.5 mL cold acetone on ice. Centrifugation was carried at 5000g for 15 min at 4 °C. Afterwards, 1mL supernatant was mixed with catalase (100 Units/mL) and subsequently kept at the room temperature for 10 min. Another 1.0 mL of supernatant as well as the blank control were mixed with 0.1 mL of 5% TiSO4 and 0.1 mL ammonia. Then the samples were centrifuged at the same rate and the pellets were re-suspended in 3mL of 2 M H2SO4, and then the absorbance was measured using UV-Visible spectrophotometer. The H2O2 contents were calculated using an external standard curve generated with known concentrations of H2O2 (0-1 µM, r2=0.998) [24, 25].

Scavenge ABTS Activity was also carried out to assess the antioxidant activity of AAS extract [25]. AAS extract (2µL) was added per well to 96-well plate, subsequently 200µL ABTS++ solution was added to each well of the plate and absorbance was measured at 745nm. Percent of scavenge ABTS was measured using following formulation. Others than percent of scavenge, Median Inhibitory Concentration (IC50) was also measured:

$$\%\text{Scavenge ABTS} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

2.5 Urease Activity

Antiulcer potential of various samples of AAS were checked through urease inhibitory activity. This activity was performed by calorimetric method on microplate reader using standard procedure.

2.6 Anti-Cholinesterase Activity

The anticholinesterase assay was performed against Acetylcholinesterase and Butyrylcholinesterase following Ellman's assay (Orhan et al. 2004). The anti-Alzheimer's disease potential of A. ascalonicum was explored following this assay.

2.7 Statistical Analysis

Every experiment was carried out at least three times, and the results were presented as

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mean ± SD. The unpaired t-test was used to analyse the comparison of two groups. To ascertain the statistical significance of treatment-related changes in survival, a one-way ANOVA analysis was carried out. *P < 0.05, **P <0.01, ***P < 0.001 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The AAS crude sample and fractions were screened by GC-MS analysis and 35 different compounds were screened which includes; Heptanal, Benzeneacetic acid, Methyl Ester, 2-Methoxy-4-vinylphenol, L-proline,5-oxy-, methyl ester, 2-amino-8-[3-d-ribouranosyl]imidazol[1,2-a]-s-triazin-4-one, Nonanedioic acid, dimethyl ester, Dodecanoic acod, methyl ester, Dihydroactinidiolide, Nonanedioic acid, dimethyl ester, Undecanoic acid, Prophylphosphonic acid, 2-oxo, methyl ester, Octadecanoic acid, 2-oxo-, methyl ester, Decanal, 3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0], 3-Hydroxy-7,8-dihydro-, beta-ionol, Hexadecanol, Methyl tetradecanoate, 1,2-Dihexylcycloprppene-3-carboxylic acid, Octadecanoic acid, 5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol, 2-cyclohexene-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butentyl), Tridecanoic acid, methyl ester, Phytol, Tetrahydrogeranylacetone, 1-Octadecyne, 10-Octadecenoic acid, methyl ester, 11- Octadecenoic acid, methyl ester, 7-Hexadecenoic acid, methyl ester, (Z)-, Hexadecenoic acid, methyl ester, 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a], Isophytol, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, methyl ester (E, E)-, 9,12, 15-Octadecadienoic acid, methyl ester, (Z,Z,Z)-, Octadecadienoic acid, and methyl ester. These isolated compounds are listed in the Table 1. These compounds have various biological and therapeutic activities, which have been further evaluated in this research work.

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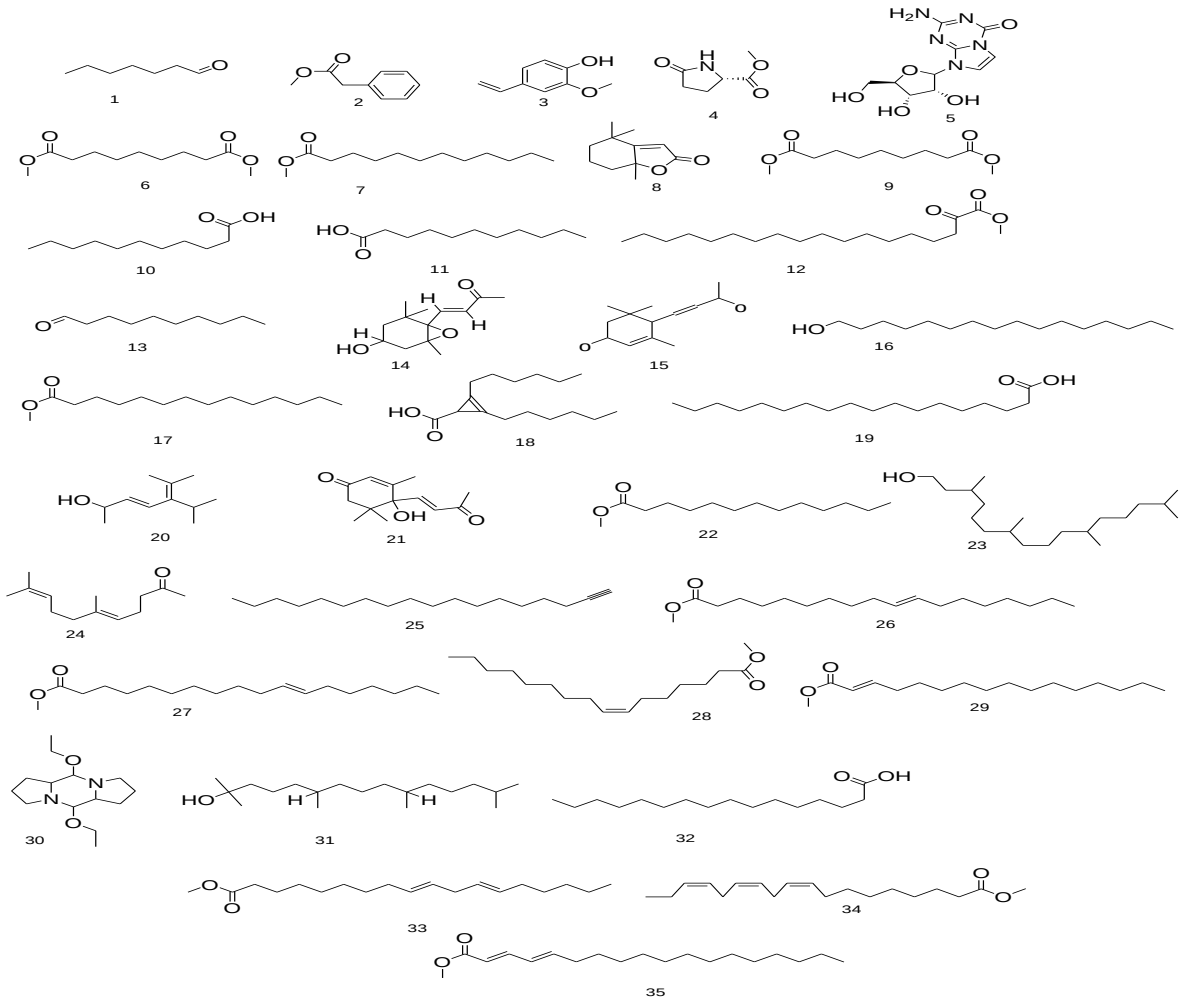

Fig 1: GC-MS Screening of AAS. The isolated compound compounds are drawn through ChemDraw

Table 1: The compounds screened through GC-MS analysis

ID#	Compound Name	R. time	Area	Conc. (%)
1.	Heptanal	5.907	22299	0.20
2.	Benzeneacetic acid, Methyl Ester	7.736	87605	0.80
3.	2-Methoxy-4-vinylphenol	10.974	61088	0.56
4.	L-proline,5-oxy-, methyl ester	12.404	100205	0.91
5.	2-amino-8-[3-d-ribouranosyl]imidazol[1,2-a]-s-triazin-4-one	13.047	13141	0.12
6.	Nonanedioic acid, dimethyl ester	13.854	18410	0.17
7.	Dodecanoic acod, methyl ester	16.054	50012	0.46
8.	Dihydroactinidiolide	16.297	33187	0.30
9.	Nonanedioic acid, dimethyl ester	16.579	11443	0.10

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10.	Undecanoic acid	16.872	7073	0.06
11.	Prophylphosphonic acid, 2-oxo, methyl ester	17.107	23073	0.21
12.	Octadecanoic acid, 2-oxo-, methyl ester	17.831	42786	0.39
13.	Decanal	18.106	8421	0.08
14.	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]	19.805	79005	0.72
15.	3-Hydroxy-7,8-dihydro-, beta-ionol	20.167	779	0.01
16.	Hexadecanol	20.381	35425	0.32
17.	Methyl tetradecanoate	20.609	121819	1.11
18.	1,2-Dihexylcycloprppene-3-carboxylic acid	20.890	27823	0.25
19.	Octadecanoic acid	21.324	23251	0.21
20.	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	21.516	430885	3.93
21.	2-cyclohexene-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butentyl)	21.985	50137	0.46
22.	Tridecanoic acid, methyl ester	22.743	51543	0.47
23.	Phytol	23.058	152977	1.39
24.	Tetrahydrogeranylacetone	23.159	101533	0.93
25.	1-Octadecyne	23.564	29065	0.26
26.	10-Octadecenoic acid, methyl ester	24.361	23502	0.21
27.	11- Octadecenoic acid, methyl ester	24.554	15833	0.14
28.	7-Hexadecenoic acid, methyl ester, (Z)-	24.696	127656	1.16
29.	Hexadecenoic acid, methyl ester	24.792	4746461	43.27
30.	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a]	24.908	30519	0.28
31.	Isophytol	25.234	28569	0.26
32.	n-Hexadecanoic acid	25.456	586026	5.34
33.	9,12-Octadecadienoic acid, methyl ester (E, E)-	28016	1117398	10.19
34.	9,12, 15-Octadecadienoic acid, methyl ester, (Z,Z,Z)-	28.144	2403531	21.91
35.	Octadecadienoic acid, methyl ester	28.624	341555	3.11

3.2 Antioxidant Activity

Oxidation reactions can deteriorate food stuff, and when occur in biological system, can then damages the cells or leading them to death. In such cases, antioxidants play a vital mighty role for the prevention of oxidation reactions. Antioxidant activity prevents cells in the physiological system as well as food materials from oxidation [24]. The antioxidant activity of AAS was determined by DPPH assay, H2O2 contents determination, and ABTS assay as reported in Figure 2A-C. The methanolic extract of AAS was used to proceed ABTS and DPPH assays. The antioxidant activity of fixed oils and ascorbic acid revealed comparably higher antioxidant activity as compared with the other isolated compounds of AAS. The IC50 values of AAS constituents are compared with control as shown in Figure 2D. In Table 2, the IC50 values of the are given for Crude AAS (Cr), EtAc, Chf, Hex, Aq, fixed oil, and ascorbic acid. Ascorbic acid was used as positive control for all the assays. The IC50 values of fixed oil and ascorbic acid were recorded 8.4±0.72, 1.54±0.02, 12.15±0.1 and 6.84, 1.66±0.013, 8.97±0.012 for ABTS, DPPH, and H_2O_2, respectively. The fixed oil has significant activity as compared to other compounds. The data proved

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fixed oil as strong antioxidant and can be utilized for clinical applications.

Table 2: IC50 value of the ABTS, DPPH and H_2O_2 assays calculated for various extracted compounds.

Compound	% ABTS IC_{50}µg/ml	% DPPH IC_{50}µg/ml	% H_2O_2 IC_{50}µg/ml
Cr	91.07±6.12	78.12±3.41	319.70±24.2
EtAc	12.58±2.13*	09.30±1.3*	52.35±2.3
Chf	26.5±2.4	21.60±2.1	61.50±4.32
Hex	86.40±6.41	56.85±6.1	314.78±14.1
Aq	106.93±4.12	91.85±7.12	143.76±5.2
Ascorbic acid	6.84±0.81	1.66±0.013	8.97±0.012

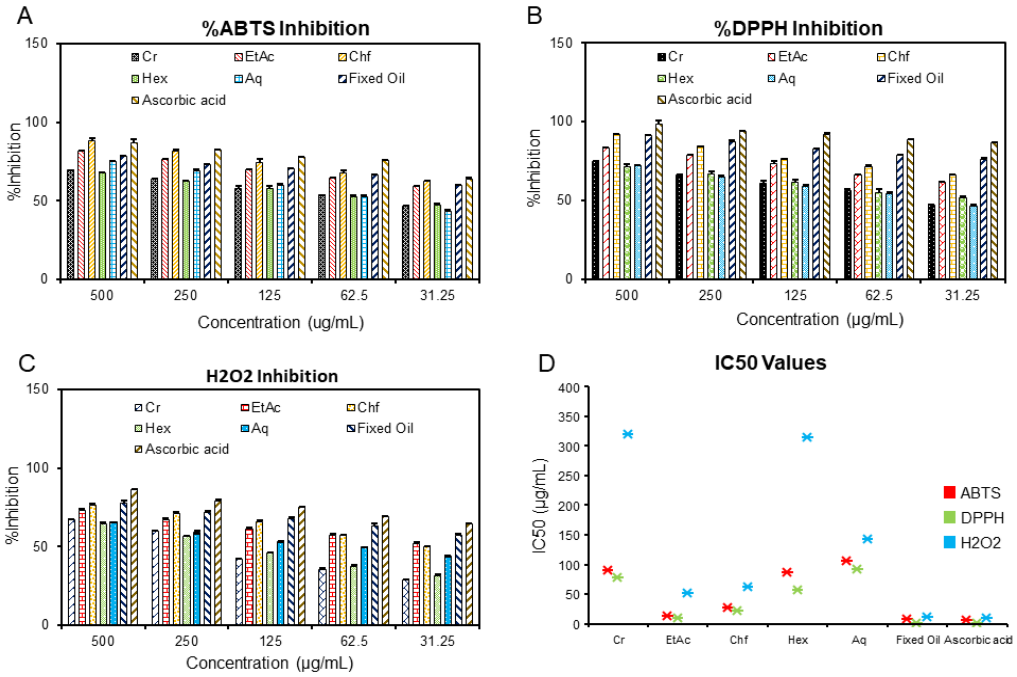

Fig 2. Antioxidant activity of AAS. A) %ABTS inhibition assay B) %DPPH inhibition assay C) H2O2 inhibition assay D) IC50 values of %ABTS inhibition assay, %DPPH inhibition assay, and H2O2 inhibition assay. Data are presented as mean ± SD, n=3. P value less than 0.05 is considered significant.

3.3 Urease Activity

In modern era, plants-derived agents that can inhibit enzymatic activities have been considered important concern in drug discovery where a number of drugs have been discovered for the treatment of various diseases. The agents/inhibitors block the enzymatic activity that can be useful in specific treatment. Here, α-glucosidase and urease are two mostly studied enzymes that are related with many disorders. α-

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glucosidase is responsible for the catalysis of starch hydrolysis by cleaving the internal α-D-glycosidic linkages and its inhibition can useful in carbohydrates metabolism related disorders like obesity, diabetes, etc. Diabetes mellitus is major global disorder characterized by hyperglycemia and α-glucosidase inhibition can be helpful in decreasing the blood glucose level.

In the current research, urease activity of isolated compounds and fixed oils of AAS was carried out and it was observed that fixed oil showed significant inhibition, while Crude AAS, EtAc and Chf also showed comparable inhibition as shown in Figure 3. It was seen that the inhibitory activity was increased with increasing of concentration of the extract. The IC50 reported for Crude, EtAc, Chf, Hex, Aq, fixed oil, and thiourea were 8.94±1.1, 12.98±1.03, 10.27±1.51, 33.34±4.3, 43.76±6.12, 5.50±0.41, and 4.30±0.23 respectively (Table 3), that thiourea was used as positive control in this study. Here, the data showed that fixed oil activity is significant, showing excellent anti-diabetic potential. These results revealed that the AAS can be new source of antienzyme drugs used for the remedy of diabetes mellitus and can be useful in decreasing the burden of diabetes around the globe.

Table 3: IC50 values of compounds for α-glucosidase inhibition assay

Compound	IC_{50}µg/ml
Crude	8.94±1.1
EtAc	12.98±1.03
Chf	10.27±1.51
Hex	33.34±4.3
Aq	43.76±6.12
Fixed Oil	5.50±0.41
Thiourea	4.30±0.23

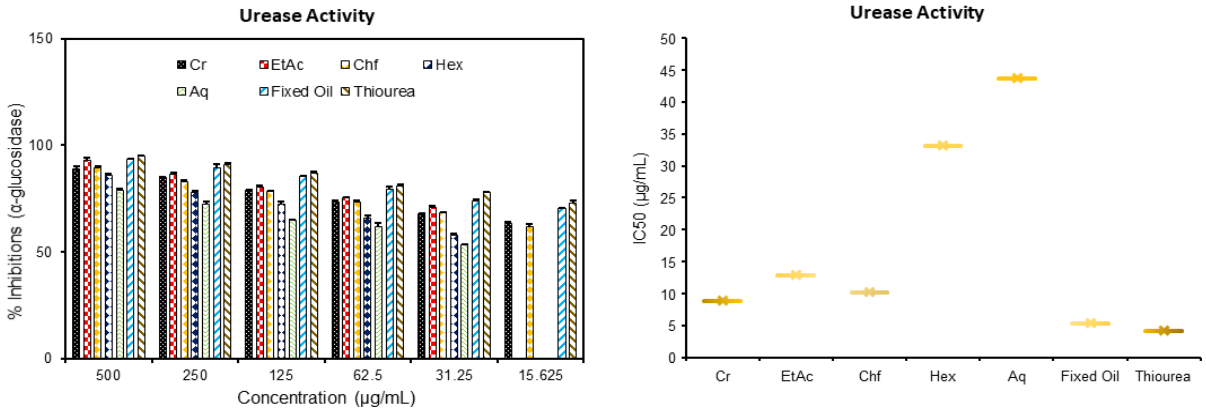

Fig 3. Urease activity of AAS extracted compounds. A) α-glucosidase inhibition of Cr, EtAc, Chf, Hex, Aq, Fixed oil, and Thiourea (control). B) IC50 values of α-glucosidase

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inhibition assay for Cr, EtAc, Chf, Hex, Aq, Fixed oil, and Thiourea (control). Data are presented as mean ± SD, n=3. P value less than 0.05 is considered significant.

3.4 Anti Cholinesterase Activity

Acetylcholine is one of the most significant neurotransmitters that has several functions in the central and peripheral nervous system. The cholinesterase inhibitors or anti-cholinesterase cause prevention of the acetylcholine breakdown. The anti-cholinesterase efficiency of the products is thought to be helpful in the treatment of alzheimer's disease. Alzheimer's disease is a major and serious problem of nervous system around the globe. The herbal medicines are thought to be good and often well accepted by the community. To test that whether the AS crude extract and its fixed oil have anti-cholinesterase activity or not, the samples were tested for AChE and BChE inhibitory activity using Ellman's method. The inhibitory activity of Cr, EtAc, Hex, Aq, Chf, and fixed Oil was compared with control group "Galantamine". The AChE and BChE inhibitory efficiencies and IC50 values are reported in Figure.

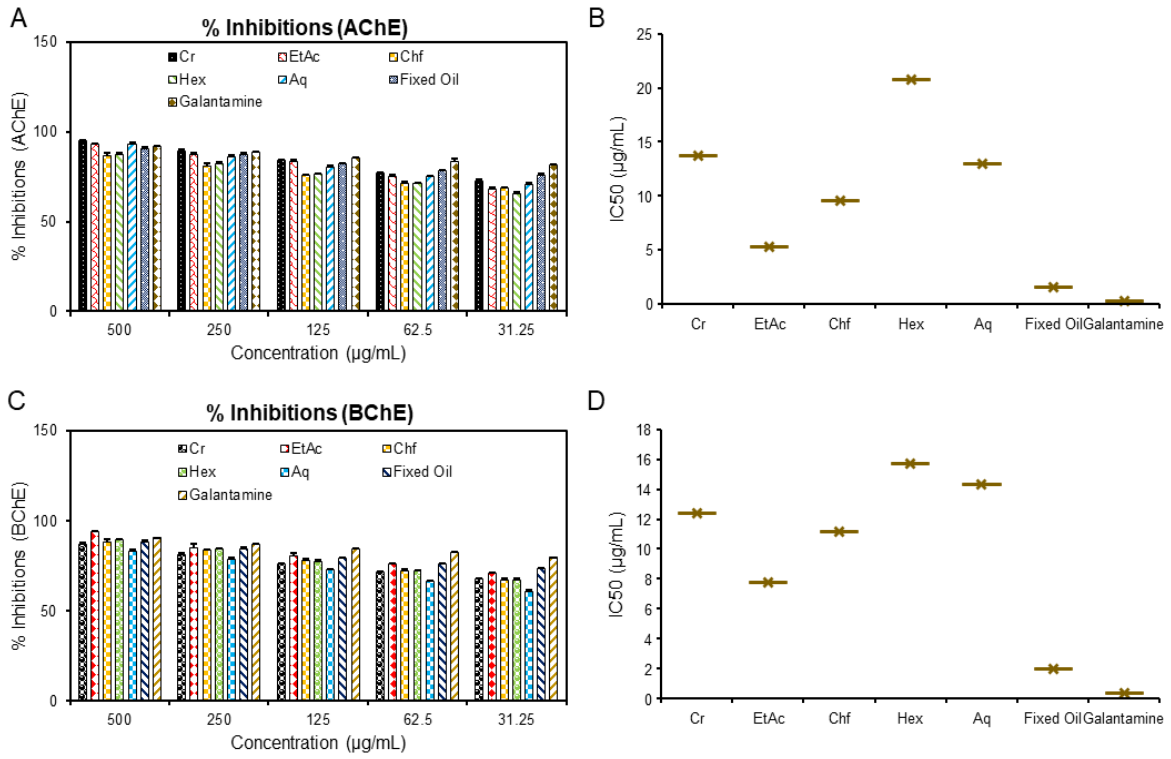

Fig 4. Anti-cholinesterase activity of AAS extracted compounds. A, B) AChE percentinhibition assay and IC50 values of Cr, EtAc, Chf, Hex, Aq, Fixed oil, and Galantamine (control). C, D) BChE percent inhibition assay and IC50 values of Cr, EtAc, Chf, Hex, Aq, Fixed oil, and Galantamine (control). Data are presented as mean ± SD, n=3. P value less than 0.05 is considered significant.

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4. CONCLUSION

The present study highlighted the ethno-pharmacological description of the AAS and provided the proof of concept for their anticancer and antimicrobial use. The plant has been already reported for various traditional therapeutic uses. Here, we isolated various compounds that has been found to be exhibiting various pharmacological activities. The data from various experiments exposed strong pharmacological effects of AAS fixed oils and other compounds. So, overall, the medicinal use of AAS against various diseases has been reflected in this study. However, more data should analyse against more pharmacological assays, to effectively use AAS Cr and fixed oils in such disorders. The current research gives an outlook for the future evaluation of AAS and AAS derived compounds including its fixed oils against various pharmacological uses.

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