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Original article

In vitro examination of anti-parasitic, anti-Alzheimer, insecticidal and cytotoxic potential of *Ajuga bracteosa* Wallich leaves extracts

Muhammad Imran [a,e], Hasnain Jan [b], Shah Faisal [c,*], Sajjad Ali Shah [c], Sumaira Shah [d], Muhammad Naeem Khan [a,f], Muhammad Taj Akbar [g], Muhammad Rizwan [h], Faheem Jan [i], Suliman Syed [c]

[a] Department of Botany, Government Post Graduate College Charsadda, KPK, Pakistan
[b] Department of Biotechnology, Quaid-i-Azam University 45320, Islamabad, Pakistan
[c] Department of Biotechnology, Bacha Khan University, Charsadda, KPK, Pakistan
[d] Department of Botany, Bacha Khan University Charsadda, KPK, Pakistan
[e] Department of Botany, Islamia College Peshawar, KPK, Pakistan
[f] Department of Biotechnology and Genetic Engineering, Agriculture University KPK, Pakistan
[g] Department of Microbiology, Abdul Wali Khan University, Mardan, KPK, Pakistan
[h] Center for Biotechnology and Microbiology University of Swat, KPK, Pakistan
[i] Programmatic Management of Drug Resistant T.B Unit Ayub Teaching Hospital, Abbotabad, Pakistan

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ABSTRACT

This research study is mainly focused to evaluate the anti-parasitic, insecticidal, cytotoxic and anti-alzheimer potential of various leaf extracts of *Ajuga bracteosa* Wallich ex Bentham. 04 different extracts were prepared using solvent of different polarity to determine the best candidate for potent bioactivity i.e. n-hexane (NH), Ethyl acetate (EA), Ethanol (EL) and Chloroform (CH). Concentrations of each extracts were made specified for all activities. All extracts were exploited for broad range of biomedical applications including leishmaniasis, in vitro anti-Alzheimer, insecticidal and cytotoxic studies. Our results showed that A. bracteosa n-hexane extract was highly active against Leishmania Tropica with significant inhibition of 58 ± 1.61 for promastigote and 63 ± 2.29 for amastigote at 1000 µg/mL. Furthermore, promising anti-alzheimer activity acetylcholinesterase (AChE) 46 ± 0.83 and butrylcholineterase (BChE) 49 ± 1.17 was noted for n-hexane. The insecticidal potential of these extracts were test against five different insects (*Rhyzopertha dominica, Trogoderma granarium, Tribolium castaneum, Sitophilus oryze,* and *Callosobruchus analis*). The higest mortality rate of insecticidal activity was recorded by n-hexane followed by Ethyl acetate whereas ethanol extract was found to be less effective against all the test species. Significant cytotoxic potential of each plant sample against *Artemia salina* thus aware us for further detailed research to find out novel drugs. Based on our results we believe that *Ajuga bracteosa* could be used to develop as a potential botanical insecticide against different insect and pests, such as aphids as well as an excellent source for the compound isolation as anti-tumor agent.

1. Introduction

Phytochemicals are bioactive chemical compounds which occur naturally in plants. "Phyto" is a Greek word which means plant. Phytochemicals present in plants are mainly accountable for developing natural immune system, provide protection for host plants and responsible for aroma, flavour and colour. To date, more than 4,000 of such compounds have been identified, and researchers are predicted to find even more. Plants have traditionally offered motivation for developing novel drug compounds, as plants derived drugs and remedies have made huge contribution to human safety, health and well-being. Their role is twofold in the development of new drugs (Tripathi et al., 2012). Secondary metabolites and natural products derived from living machineries, especially plant-based, have demonstrated great potential in the treatment of human diseases such as cancer, heart disease, diabetes and infectious diseases (Chew et al., 2011). Natural products have influ-

* Corresponding author.
E-mail address: shahfaisal11495@gmail.com (S. Faisal).
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enced several discoveries in organic chemistry leading to advancements in synthetic methodologies and in the production of many analogues of therapeutically potentially lead compounds. Cinchona 's approval for the treatment of malaria in the 17th century, preceded by digitalis, morphine, etc., and the advent of aspirin, led the general population to believe in the miracles of abundant floral resources, and that natural goods, like plants, provide considerable systemic variety for the pharmacological treatment of specific groups of disorders (Mukherjee et al., 2010).

Ajuga bracteosa Wallich ex Bentham is a member of family *Labiatae* of about 180 genera and more than 3500 species. Majority members of the family are aromatic, annual to perennial, herbs or shrubs (Ali, 2008). Traditionally the plant is used for curing malaria and gout and is consider being an alternative for cinchona (Pal and Pawar, 2011). It is used as blood purifier, blood cooling agent, in treatment of dyspepsia, hyper tension, sore throat, chest pain and fever (Hazrat et al., 2011; Ibrar et al., 2007; Murad et al., 2011; Sher, 2011). Besides these different traditional usage, *Ajuga bracteosa* is well known for their significant anti-inflammatory activity, antibacterial activity and anti-plasmodial activity (Gautam et al., 2011; Hsieh et al., 2011; Vohra and Kaur, 2011). A large number of metabolites have also been extracted from the genus *Ajuga*, majority of which are biologically active. These chemicals include phytoecdysteroids, *neo*-clerodane-diterpenes, diterpenoids, triterpenes, sterols, anthocyanidin-glucosides, iridoid glycosides, withanolides, flavonoids and triglycerides (Coll and Tandrón, 2005; Israili and Lyoussi, 2009; Singh et al., 2010).

Several medicinal plants or their products have been tested against antibiotic resistant pathogenic microbes (Haraguchi et al., 1999). Medicinal plants or their products are used for treatment of diseases all over the world. According to the report of WHO (2002), 70–80|% population of both developed and developing countries uses traditional medicines, mostly of plant origin for treatment of diseases (Ahmad et al., 2010; Shrestha and Dhillion, 2003; Usman et al., 2020). Use of medicinal plants in food products boost up natural immunity of the body against various disease causing agents due to the presence of high level of phytochemicals, such as carotenoids, flavonoids, phenolics and terpenoids (Conforti et al., 2008; Hudaib et al., 2008; Jänicke et al., 1998). These phytochemicals have been reported to perform different biological activities, e.g. anticancer (Chander, 2018), anti-inflammatory (Zhang and Tsao, 2016), antibacterial (Gohain et al., 2019), antiviral (Orhan et al., 2012), anti-ischemic (Pozdnyakov et al., 2019) and vasodilator (Luna-Vázquez et al., 2018). In addition, they may inhibit lipid peroxidation and platelet aggregation, and increase capillary permeability and fragility (Smeriglio et al., 2019).

Plant based botanical insecticides are comparatively better alternatives to synthetic chemical insecticides for pest management because they pose are environment and human health friendly (Isman, 2006). All over the world scientists are trying to develop plant based pesticides, because the commercial pesticides negatively affect the environment as well as human health (Tariq et al., 2010). After severe setback and hazardous effects of chemical pesticides on living systems and on the environment, the use of eco-friendly bio-pesticides is getting increased (Kumar et al., 2011). Similarly, there is a need to develop herbal drugs for curing cancerous diseases, because most of the synthetic drugs used for the treatment of cancer have severe side effects. Cytotoxicity can be best studied through brine shrimp bioassay because toxicity to brine shrimps has good relation with anti-tumor in humans (Demirgan et al., 2016).

To the best of our knowledge, it is the first ever report on biological analysis of medicinally important *Ajuga bracteosa*. Different types of extracts (N-hexane, chloroform, ethanol and ethyl acetate) were prepared using leafs of *A. bracteosa* and were investigated for their clinical and biological applications.

2. Materials and methods

2.1. Plant collection and extracts preparation

In the current study the herb has been obtained from District Charsadda, Khyber Pakhtunkhwa, Pakistan. The plant was taxonomically identified by the Department of Botany, Bacha Khan University, Charsadda as *Ajuga bracteosa*.

The fresh leaves were excised with a sterile surgical blade into tiny pieces, rinsed well with distilled water to eliminate any contaminants and impurities of soil, accompanied by drying in daylight. The well-dried leaves in a Willy mill were then ground into fine powder, and processed for aqueous extraction at 25 °C. n-hexane, ethanol, chloroform and ethyl acetate leaf extract of plant material were prepared by inserting 25 g of fine powder in flasks (500 mL) comprising 200 mL of respective solvent, continuously sonicated for 10 min, and held at 40 °C for two days in a shaking incubator at 200 rpm. The prepared extracts were initially filtered twice with nylon cloth to remove solid residues and the obtained extracts were further filtered using Whatman filter paper three times to remove any remaining particulates. The fresh filtrate was then dried and processed for further use.

2.2. Anti-leishmanial assay

The anti-leishmanial potential of the plant extracts was assessed against the amastigote and promastigote cultures of *L. tropica* KWH23 (Department of Biotechnology IIUI Pakistan) (Ahmad et al., 2015; Jan et al., 2020a; Jan et al., 2020b). M199 media having 10% fetal bovine serum helped for culturing leishmanial parasites. *Leishmania* culture at a density of 1×10^6 cells/ml was used for the analysis. The activity was executed in a 96-well plate with concentration ranging from 10 to 1000 μg/mL. DMSO was used as blank and Amphotericin was used as a positive control. The seeded 96-well plate with test dilutions were incubated at room temperature for 72 hrs. OD was noted at 540 nm, while all the lived cultures were counted using an inverted microscope and their LC50 values were calculated by applying Table curve software.

$$\%\text{Inhibition} = \left[1 - \left\{\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right\}\right] \times 100$$

2.3. Insecticidal activity:

Insecticidal activity of the crude extract was carried out by impregnated filter paper method following (Tariq et al., 2010). Five grain pests (*Trogoderma granarium, Callosobruchus analis, Sitophilus oryzea, Tribolium castaneum* and *Rhyzopertha dominica*) were reared in laboratory under controlled conditions (temperature and humidity) in plastic bottles having sterile breeding media. Insects of uniform age and size were used for the experiment. Filter papers were cut according to the size of Petri plate (9 cm) and put in the plates. Three concentrations. i.e. 10, 100 and 1000 μg/mL were made from the stock solution. A 10 mL of each concentration was then poured on separate filter papers in Petri plates. The plates were left open to evaporate the solvent completely. 10 healthy and active insects of the same size and age of each species were put in each plate with the help of a clean brush. Permethrin was used as positive control while DMSO was used as negative control. The plates were incubated at 27 °C for 24 h. On the third day readings

were recorded and the percentage inhibition or percentage mortality with the help of the following formula was calculated:

$$PercentMortality = 100 - \frac{No.ofinsectsalive intest}{No.insectsalive incontrol} \times 100$$

2.4. Cytotoxicity screening

The cytotoxic activity of different extract of *Ajuga bracteosa* was carried out by following the method of (Meyer et al., 1982). The material and reagents needed for the activity included eggs of *Artemia salina* and sea salt. 38 g of sea salt was dissolved in one liter of distilled water and the pH was adjusted to 7.40. For hatching of brine shrimp eggs, filtered brine solution was taken in a rectangular hatching tray and about 50 mg of shrimps eggs were sprinkled over it and incubated at 37 ^{0}C for 24 h to obtain larvae. 10 mg of the test extract was dissolved in 1 mL of respective solvents, that was used as stock solution and from this stock solution 10, 100 and 1000 μg/ mL concentration were prepared by taking 10, 100 and 1000 μL of the stock solution respectively in small vials. Five replicates of each concentration were made. The vials were kept open to evaporate the solvent. After 2 days of hatching and maturation, 10 larvae/vial were placed, using a pastuer pipette. The volume was made 10 mL by adding seawater. These were then incubated at (25–27 ^{0}C) for 24 h under illumination. In other vials DMSO and standard cytotoxic drug were taken which served as negative and positive controls respectively.

2.5. Anti-Alzheimer activity

Anti-alzheimers activity of plant extracts were measured by their ability to inhibit acetylcholinesterase (AChE; Sigma ‘‘101292679: 0.03 U/mL) and butyrylcholinesterase (BChE; Sigma ‘‘101303874: 0.01 U/mL) (Faisal et al., 2020). In brief, plant extracts were dispersed in Phosphate Buffer Saline (PBS) with concentration ranging from 10 μg/mL to 1000 μg/mL. A substrate solution was prepared in dH_2O that constituted butyrylcholine iodide (BTchI; 0.0005 M), DTNB (5, 5-dithiobisnitrobenzoic acid; 0.00022 M), and acetylcholine iodide (ATchI; 0.0005 M). In the assay, pristine reaction mixture and Galanthamine hydrobromide (5 mg/0.5 mL methanol) were used as positive and negative controls, respectively. The principle of the assay is based on ATchI and BTchI hydrolysis by AChE and BChE, respectively leading to 5- thio-2- nitrobenzoate anion formation that gives yellow colour when form complexes with DTNB. Finally absorbance of the samples were recorded with UV–VIS spectrophotometer at 412 nm. The % enzyme activity and enzyme inhibition activities were calculated as

$$\%\text{Enzymeactivity} = \left(\frac{V}{Vmax}\right) \times 100$$

$$\%\text{Enzymeinhibition} = 100 - \%Enzymeactivity$$

3. Results

3.1. Plant identification and extraction

Ajuga bracteosa leaves were collected from District Charsadda, KPK Pakistan. The plant identification was carried by Professor Imtyaz Department of botany, Bacha Khan University Pakistan. The plant leaves were cut into small pieces, rinsed with distilled water twice and then with distilled water to eliminate impurities and dust spores, followed by drying in the shade. Dried leaves were then fine powdered in a Willy mill and 30 g of fine powder were added into flasks (500 mL) containing 200 mL of distilled water, methanol, ethanol and ethyl acetate respectively, continuously sonicated for 10 min and held at 37 °C for two days in a shaking incubator at 200 rpm. The prepared extract were initially filtered twice using nylon paper, and was then filtered three times using Whatman filter paper to eliminate all residual and solid matter.

3.2. Anti-leishmanial activity

Leshmaniasis, is highly neglected, non-contagious tropical and subtropical infectious disease caused by parasites largely found in *Leishmania* species. According to World health organization (WHO), the disease is endemic in 89 countries and with annual global incidence of 1.5 to 2 million cases worldwide. The disease is caused by an intracellular parasite and are transmitted to humans by sand flies (Phlebotomus and Lutzomyia) bite. Due to inappropriate vector and inefficient and affordable drugs the disease is at high risk of uncontrolled spreading. In our study, different plant extracts concentrations of 10, 100 and 1000 μg/mL were investigated against both promastigote and amastigote cultures of *L. tropica* via MTT assay as shown in Fig. 1. As can be observed, a dose dependent cytotoxicity was observed with significant mortality rate of 63.18 ± 2.29 and 58.44 ± 1.61 for n-hexane, 57.20 ± 1.19 and 48.39 ± 1.09 for ethanol at 1000 μg/mL for promastigote and amastigotes form of the parasite, respectively. Moreover, moderate values were observed for both the patristic forms i.e. as 39.40 ± 0.69 for promastigote and 44.19 ± 0.71 for amastigote by ethyle acetate respectively.

3.3. Insecticidal assay

The insecticidal potential of different plant extracts was tested for 5 different insects i.e. *Trogoderma granarium, Callosobruchus analis, Sitophilus oryzea, Tribolium castaneum* and *Rhyzopertha dominica* are shown in Table 1. Significant mortality values were recorded for all the extract at higher concentration 1000 μg/mL, 100 μg/mL and 10 μg/mL respectively against all the test insects. Among the different tested insects *S. oryzea* was found to be the most susceptible insect to all the extract showing a % mortality (64%, 50% and 42%) by n-hexane followed by chloroform (62%, 48% and 40%) and ethanolic extract (60%, 46% and 38%) respectively. In contrast the lowest % mortality was recorded for ethyle acetate i.e. (56%, 44% and 42%), at 1000, 100 and 10 μg/mL conce-

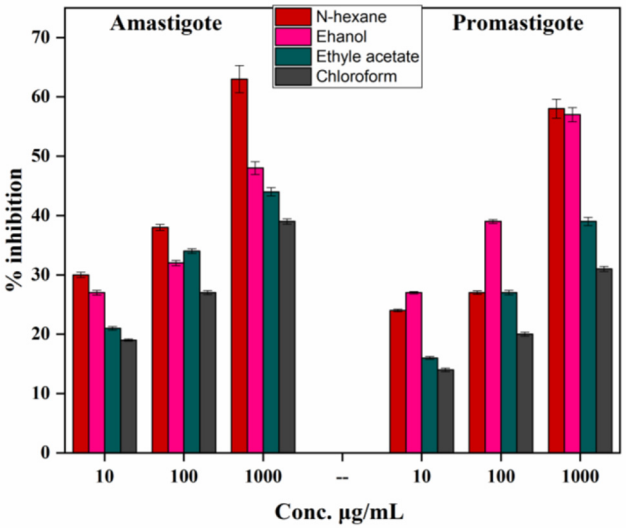

Fig. 1. Anti-leishmanial potential of different extracts of *Ajuga bracteosa.*

Table 1
Insecticidal potency of *Ajuga bracteosa* leaf extracts.

Test species	Extracts	Concentration		
		10 μg/mL	100 μg/mL	1000 μg/mL
S. oryzea	NH	42	50	64
	EL	38	46	60
	CH	40	48	62
	EA	42	44	56
T. castaneum	NH	43	43	66
	EL	31	40	54
	CH	40	50	64
	EA	32	54	60
C. analis	NH	34	52	64
	EL	30	48	58
	CH	32	50	60
	EA	36	54	64
T. granarium	NH	26	34	40
	EL	20	30	36
	CH	22	32	38
	EA	28	36	44
R. dominica	NH	29	35	46
	EL	32	39	41
	CH	29	33	44
	EA	37	37	47

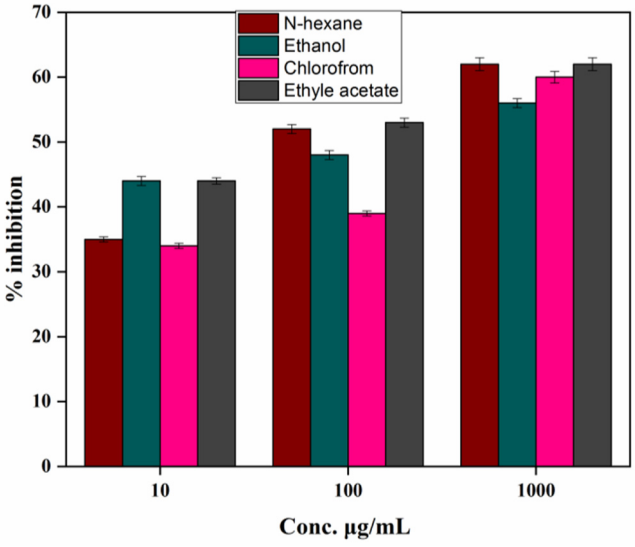

Fig. 2. Cytotoxic potential of different extracts against *Artemia salina.*

trations. Beside *S. oryzea* the next highly susceptible specie was *T. castaneum* which showed (66%, 43% and 43%) for n-hexane followed by chloroform (64%, 50% and 40%) and ethyle acetate was noticed for its minimum % mortality (60%, 54% and 32%) respectively. Similarly the *C. analis* inhibition was recorded as (64%, 54% and 36%) for ethlye acetate followed by n-hexane (62%, 52% and 34%), chloroform (60%, 50% and 32%) and ethanol (58%, 48% and 30%) for same concentrations respectively. The least effected test specie was *T. granarium* almost to all extracts followed by *R. dominica* with minimum mortality rate. In case of *T. granarium* i.e ethyle acetate showed (44%, 36% and 28%) followed by n-hexane (40%, 34% and 26%) follwed by chloroform (38%, 32% and 22%) and the least activity was recorded for ehhanol extract (36%, 30% and 20%) respectively. The overall manner of inhibition against the different test insects was recorded as *S. oryzea* > *T. castaneum* > *C. analis* > *R. dominica* > *T. granarium*.

3.4. Cytotoxic activity

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. Based on the results, all the extracts of *Ajuga bracteosa* to brine *shrimp nauplii* showed significant cytotoxic activity. Maximum activity was showed by ethyl acetate (44.26 ± 0.49, 53.33 ± 0.69, 62 ± 1.08) inhibitions at 10, 100 and 1000 μg/mL followed by ethanolic extract (44.31 ± 0.71, 48.0 ± 0.77 and 56.0 ± 0.79). Inhibition for n-hexane was (35.33 ± 0.49, 52.0 ± 0.70, 62.67 ± 1.04) however the least inhibition was recorded for chloroform i.e. (34.67 ± 0.41, 39.33 ± 0.40, 60.67 ± 0.89) at same concentrations Fig. 2. Our results confirmed that, the degree of lethality was to be directly proportional to the concentration of the extract.

3.5. Anti-Alzheimer assay

In the study, different concentrations of the plant extracts were probed for inhibition response of two cholinesterase enzymes i.e. Acetylcholinesterase (AChE) and butrylcholineterase (BChE) (Khalil et al., 2018). Interestingly, the inhibition response for both esterases was dose dependent. Ethanol were most active at 1000 μg/mL resulted in 56.51 ± 0.48% inhibition of AChE and 52.49 ± 0.50% for BChE, followed by N-hexane resulted in 51.37 ± 0.50% for AChE and 55.41 ± 0.44% for BChE respectively. While lower inhibition response of AChE 44.65 ± 0.44% and BChE 49.13 ± 0.44% for chloroform at 1000 μg/mL was observed. Overall, plant extracts were found highly active against both the enzymes as indicated by their values for AChE and BChE in Fig. 3. Our results are matching with previous studies (Bahadori et al., 2017; Uddin et al., 2019).

4. Discussion

The present research was conducted to investigate the insecticidal, anti-parasitic, anti-alhzeimer and cytotoxic activities of the various extracts i.e. NH, CH, EA and EL of the *Ajuga bracteosa.* All the extracts were proven to have a significant insecticidal potential against the test insect at higher doses of 1000, 100 and 10 μg/mL. Recently plant based products and phytochemicals based treatments have gained much attention owing to their significant cytotoxicity against Leishmania (Jebali and Kazemi, 2013). However, some of medicinal plants with different polarity extracts have been rarely explored for their cytotoxic potential against the parasite: *Leishmania tropica* (KWH23). Results of extracts formulations in this study were found to have significant inhibition of 63.18 ± 2.29 and 58.44 ± 1.61 for n-hexane, 57.20 ± 1.19 and 48.39 ± 1.09 for ethanol at 1000 μg/mL for promastigote and amastigotes form of the parasite, respectively. Moreover, moderate values were observed for both the patristic forms i.e. as 39.40 ± 0.69 for promastigote and 44.19 ± 0.71 for amastigote by ethyle acetate respectively. The results suggests that *A. bracteosa* extracts can be employed as an important tool for the futuristic anti-leishmanial therapies. Our findings, are in agreement with some of the previous studies reported and thus emphasizes on the futuristic possibilities for medicinal plants as an alternative to current anti-leishmanial therapies (Mans et al., 2016; Nikmehr et al., 2014).

Overall the rate of mortality was directly proportional to the extract concentration i.e. as we increased the extract dose from lower to higher the % mortality was also got increased. The major findings of our conducted study indicates the presence of botanical insecticidal potentioal of *Ajuga bracteosa.* All the tested insects are known to produce massive damage to standing crops as well as to stored grains so their control is also important. A similar research work was also conducted by different researchers for the investigation of plants based insecticides (Jbilou et al., 2006; Khan et al., 2017). Among the different tested insects *S. oryzea* was found to

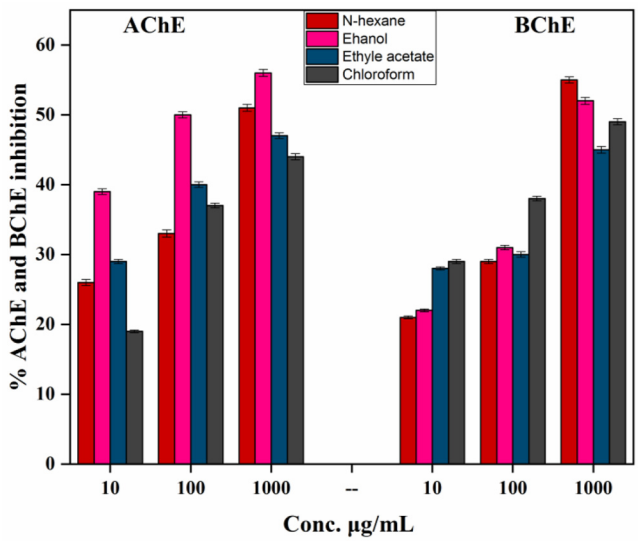

Fig. 3. Anti-AChE and Anti-BChE potential of *Ajuga bracteosa* extracts.

be the most susceptible insect to all the extract showing a % mortality (64%, 50% and 42%) by n-hexane followed by chloroform (62%, 48% and 40%) and ethanolic extract (60%, 46% and 38%) respectively. In contrast the lowest % mortality was recorded for ethyle acetate i.e. (56%, 44% and 42%), at 1000, 100 and 10 µg/mL concetrations. Beside *S. oryzea* the next highly susceptible specie was *T. castaneum* which showed (66%, 43% and 43%) for n-hexane followed by chloroform (64%, 50% and 40%) and ethyle acetate was noticed for its minimum % mortality (60%, 54% and 32%) respectively.

Rahman and his colleagues investigated insecticidal activity of *Sapindus mukorossi* ethanolic extract against *Sitophilus oryzae* and *Pediculus humanus*. Their results indicated that the toxic and repellent effect of the plant was proportional to the concentration and higher concentration has stronger effect. They found that mortality percentage increased with increase in time intervals after treatment which is an agreement with our research work (Rahman et al., 2007). Similarly a group of researchers conducted a study on the seed extracts of *Annona squamosa* L in petroleum spirit, ethyl acetate, acetone and methanol against *Tribolium castaneum*. Among the different extracts petroleum spirit extract offered highest toxicity to word the test insect and provide support for our results (Khalequzzaman and Sultana, 2006). A similar study was conducted to examine the insecticidal potential of crude extracts of *Tagetes erecta* Linn against *Tribolium castaneum*. Their results revealed that the chloroform extract had highest toxicity against both the larvae and adults of *Tribolium castaneum* followed by petroleum ether extract and ethanol extract showing consistence to our findings (Nikkon et al., 2009).

The different extracts of the same plant *Ajuga bracteosa* were evaluated for its cytotoxic activity using brine *shrimp nauplii* eggs as a test object. The main findings of the current work reveals that % toxicity shown by ethyle acetate was (44.26 ± 0.49, 53.33 ± 0.69, 62 ± 1.08) at doses of 10, 100 and 1000 µg/mL followed by EL, NH and CH respectively. Here % mortality was also dose dependant . These results support the confirmation of active cytotoxic substances in *Ajuga bracteosa*. The searching of plant based active anticancer agents has been successful throughout the world which are nowadays uses for curing the tumors. In order to evaluate such active substances various researchers conducted researcher in same domain (Hamidi et al., 2014; Ohikhena et al., 2016).

Alzheimer's diseases (AD) is a progressive neurodegenerative disease contributing to 60–80% of dementia cases worldwide. The disease is characterized by gradual decline in cognitive abilities such as memory, executive and visual spatial functioning, personality and language. The prevalence rate of the disease are alarming and in United States alone a person develops AD every 65 s (Weller and Budson, 2018). Current treatments available for AD include cholinesterase inhibitors for patients with any stage of AD. Diverse synthetic and natural substances have been reported for the effective inhibition of cholinesterase enzymes. The enzymes functions by catalyzing the hydrolysis of acetyl choline (neurotransmitter) into choline and acetic acid in the synapsis or neuro-muscular junctions with in the tissues. The decreased levels of acetyl choline results in progression of AD. In the study, different concentrations of the plant extracts were probed for inhibition response of two cholinesterase enzymes i.e. Acetylcholinesterase (AChE) and butrylcholineterase (BChE) (Khalil et al., 2018). Interestingly, the inhibition response for both esterases was dose dependent. Ethanol were most active at 1000 µg/mL resulted in 56.51 ± 0.48% inhibition of AChE and 52.49 ± 0.50% for BChE, followed by n-hexane resulted in 51.37 ± 0.50% for AChE and 55.41 ± 0.44% for BChE respectively. While lower inhibition response of AChE 44.65 ± 0.44% and BChE 49.13 ± 0.44% for chloroform at 1000 µg/mL was observed. Overall, plant extracts were found highly active against both the enzymes as indicated by their values for AChE and BChE. Our results are matching with previous studies (Bahadori et al., 2017; Uddin et al., 2019).

5. Conclusion

Our results directed us to the deduction that extraction efficacy, biological efficiency and nature of pharmacological response are reliant on the solvent type and its polarity. Better results can be attained if a multi range polarity based solvent system is employed in the preliminary screening stages monitored by optimization of the extracts and bioactivity guided isolation of potentially active fractions accountable for the observed activity. The results of the present study revealed that *Ajuga bracteosa* possesses constituents that are capable of exhibiting various bioactivities. Further work is required to identify and isolate the active constituents of the plant, which may be helpful in developing herbicides, insecticides and cytotoxic agents of natural origin and possibly could be best alternative for synthetic chemicals used for these purposes with minimum side effects. From the above-mentioned biological potential, it has been concluded that these extracts could be suitable candidates for various biomedical research and application.

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Declaration of Competing Interest

The authors declared no competing interest.

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