Journal of Population Therapeutics & Clinical Pharmacology

Research Article
DOI: 10.53555/m43wjc22

EVALUATING THE ANTI-ALZHEIMER POTENTIAL OF VARIOUS PLANTS'S EXTRACTS AND THEIR FRACTIONS THROUGH MODULATION OF INSULIN FIBRILLATION

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ABSTRACT

Background: The susceptibility of protein structures to form aggregates under unsuitable conditions from their native state gives rise to various diseases, including Alzheimer's disease. In the brain, the conversion of protein aggregation into amyloid fibrils is the hallmark of Alzheimer's pathology; therefore, suppressing amyloid fibril formation is the best approach for treating Alzheimer's disease. The suppression of fibril formation and blockage of fibril aggregation are therapeutic approaches to combat such neurological diseases. Three plants—*Curcuma longa* (Haldi), *Emblica officinalis* (Amla), and *Piper nigrum* (black pepper)—have been commonly utilized to prevent and treat various diseases for centuries. The goal of this research is to determine and correlate anti-insulin fibrillation, as well as to correlate the anti-Alzheimer's potential of phytochemicals from crude methanolic extracts and their fractions of the three selected plants.

Methodology: Dried plant materials were extracted in methanol for 22 days. After filtration,the methanol was evaporated using a rotary evaporator under reduced pressure to obtain crude extracts. These extracts were then fractionated with hexane, ethyl acetate, and butanol. The fractions were concentrated under reduced pressure. Primary screening for bioactive compounds was performed using TLC, and anti-insulin fibrillation activity was assessed using the Thioflavin T assay, with insulin as a model protein.

Results: According to TLC analysis using the ethyl acetate solvent system, all three plants showed active components, while using the methanol solvent system, all three plants showed active components except crude methanolic extract of Piper nigrum. In the hexane solvent system, only the crude methanolic extract of *Curcuma longa* and its hexane and ethyl acetate fractions showed active components, while in the chloroform solvent system, *Emblica officinalis* did not show any active components. According to the Thioflavin T assay, the crude methanolic extract of *Curcuma longa* exhibited the highest activity, with IC_{50} values of 0.55 µg/ml, whereas the ethyl acetate extract of *Curcuma longa* showed the lowest activity, with an IC50 value of 41.52 µg/ml against insulin fibrillation.

Conclusion: Based on TLC and ThT assay results, our findings demonstrate that the crude methanolic extract of *Curcuma longa* suppresses amyloid formation and exhibits enhanced potential compared to the other plants tested in this study, suggesting its potential in the treatment of Alzheimer's disease.

Keywords: Alzheimer disease, Curcuma longa, Amyloid fibril, Insulin fibrillation, Plants extracts, Thin layer chromatography, ThT assay, Medicinal plants.

1. INTRODUCTION

Alzheimer's disease is a neurological disorder characterized by the deterioration of memory and cognitive processes. The etiology of Alzheimer's disease includes an overabundance of amyloid-β protein, lipid peroxidation, ventricular hypertrophy, degeneration of cholinergic neurons, posterior cortical atrophy (PCA), and a deficit of acetylcholine (ACh) (Ahmadi, et al.,2021). According to the WHO, the recognition rate of Alzheimer's disease is expected to rise by 0.556% by 2030, affecting individuals aged 65 and above (Dey, et al., 2017). As reported by the Alzheimer's International Association, by 2050, 115.4 million people worldwide will suffer from Alzheimer's disease. However, no effective treatment is available to date. Hence, it is significant to illustrate the processes that cause Alzheimer's disease and to identify remedial approaches for its therapy (Zhou, et al.,2017). The displacement of Aβ peptide (Amyloid β peptide) and the aggregation of Aβ proteins in the brain are fundamental markers in patients suffering from Alzheimer's disease (Wakabayashi, et al., 2019).

Amyloid fibrils are self-developed protein aggregates in Alzheimer's diseases (Ow, and Dunstan, 2014).Under stress, these proteins can become misfolded, causing monomers to conform into amyloid fibrils or amorphous protein aggregates. (Debnath, et al., 2017). Comprehensive study of protein aggregation provide a plan in resolution of likely antagonists or remedy in neurological disorders, which impact thousands of people globally. Many protein aggregation experiments being organized both *in vitro* and *in vivo methods*.Out of which, *In vitro* is a rapid method, easily manipulable under conditions such as agitation or increased temperature (Ziaunys, and Smirnova, 2019).

Insulin is utilized as a model for an amyloid aggregation inhibitor in *in vitro* models. The native structure of insulin is related to amyloidogenic proteins like lysozyme and prion. Amyloid insulin has a cross-β arrangement, similar to amyloid fibrils, and therefore, is applicable in the research studies of aggregation inhibitors (Gong, et al., 2014). Human insulin is a hormone with 51 residues that play role in maintaining glucose levels in blood. Originally, insulin has a helical structure. Under high temperatures or in strong acid, monomers of insulin combine to form fibrils (Li, et al., 2019). While in a passive state , incubated insulin does not produce amyloid fibrils (Long, et al., 2019). At acidic pH 2, and temperature 65°C, for 12 hours, incubated insulin altered to produce amyloid fibril (Jayamani, and Shanmugam, 2014). Fibrils grow at acidic pH,. Fibrils formation depends on various factors such as temperature, pH and agitation etc. (Rosetti, and Marchesan, 2023).Different studies have demonstrated that various active compounds from plants, nanoparticles, artificial antioxidants, and peptides are effective in blocking amyloid fibril production, However, Investigation on amyloid diseases still remains incomplete (Gomathi, et al., 2021).

Curcuma longa (Zingiberaceae), commonly called turmeric are "spice of life" frequently famous as "golden spice". (Agarwal, et al., 2018). It exist in South Asia such as Pakistan, Bangladesh, china, Indonesia and Sri lanka(Rezaei, et al., 2023). Perennial plant and rhizome are tubular and subsidiary (Fuloria, et al., 2022). Rhizome comprise of protein 6.3%, carbohydrates 69.4%, minerals 3.5% and fat 5.1%. Curcuminoids [i.e. Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin] and volatile oil are main components exist in rhizome. Curcuminoids accountable for yellow colour and. volatile oil accountable for fragrance (Dosoky, et al., 2018).Rhizome are used in reduction of inflammation, cancer, microbial infection, diabetes, oxidation and Alzheimer's (Momenkiaei, and Raofie, 2019).Curcuminoids has significant anti-Alzheimer's activity by decreasing Amyloid plaques (Ahmed, and Gilani, 2014).

Emblica officinalis (Euphorbiaceae), commonly called Amla (Pawar, et al., 2022).are recognized as the "Fruit of the twenty-first century" and the "Wonder Fruit of health" due to its specific effects (Singh, et al., 2022).It is found in India, Malaysia, Pakistan, Srilanka, China, Uzbekistan,Vietnam and Thailand. Plants are shortened upto 5.5 m, Fruit are orbiculate, flushy and heavy cup shaped

(Baliga, et al., 2013). Amla fruit contains large amount of vitamin C, proteins, tannins, amino acids, fibers, polyphenols, and minerals (Kapoor, et al., 2020). Higher vitamin C and polyphenols are responsible for its antioxidant activity. Other benefits include discourages of lipid accumulation, inflammation, diabetes, cancer, neurological and GIT disorder (Gul, et al., 2022). Amla fruit powder has significant effect on $AlCl_3$ induced Alzheimer in rats by antioxidant activity (Vishala, et al., 2019). *Piper nigrum* (Piperaceae) also known Black pepper Plant are woody perennial. Native spice of cultured globally such as Pakistan, Africa, America (south) and India(south-west). (Tasleem, et al., 2014). King of spice in world because of its peppery nature(Ahmad, et al.,2012). Main alkaloid in black pepper i.e. Piperine responsible for odour. (Tasleem, et al., 2014). Piperine has medicinal values such as fever reduction, pain relief, antibacterial and activation of the nervous system Pyrrolidine and piperidine occur in black pepper (Reddy, et al., 2004). Black pepper volatile oil has carminative, antimicrobial, antioxidant and antifungal action. Major compounds of volatile oil are limonene, sabinene, α and β pinene, 3-carene and β-caryophyllene (Dosoky, et al., 2019).Piperine in black pepper inhibit amyloid fibril and acetylcholine esterase helpful in neurodegenerative diseases like Alzheimer's (Sharma, et al., 2023).

The current investigation was designed to compare the effect of these three plants methanolic extracts and their fractions on insulin fibrillation to determine their anti Alzheimer's activity.

2. MATERIALS & METHODS

2.1 Chemicals and reagents

All chemicals and solvents used in our experimental studies were of analytical grade and were obtained from Merck KGaA, Darmstadt, Germany. Thioflavin T (ThT) and Recombinant human insulin was purchased from Sigma-Aldrich, USA. TLC plate along with silica gel 60 F_{254} a were sourced from Merck KGaA, Darmstadt, Germany.

2.2 Plant material

The dried rhizome of *Curcuma longa* (C), dried fruits of *Embelica Officinalis* (E), and dried fruits of *Piper nigrum* (B) were obtained from the regional market in Karachi, Pakistan. Taxonomic analysis of these herbs was confirmed by Dr. Ghazala (Department of Pharmacognosy, University of Karachi, Karachi, Pakistan), and voucher specimens numbered *Curcuma longa* (109 A), *Embelica Officinalis* (42 A) and *Piper nigrum* (70 A) were deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.

2.3 Extraction of plant materials

One kilogram of plant material (C, E, B) was extracted separately in 99.5% methanol (Qiao, et al., 2019; Larson, et al., 2016) for a period of twenty-two days. The filtrate was then evaporated on a water bath using a rotary evaporator under reduced pressure (Buchi rotavapor model R-200 with model B-490 water heating bath, Switzerland) to obtain the crude methanol extract with a brown pigment (30 g of C-6, 60 g of E-6, 22.5 g of B-6) (Ali, et al., 2013). The crude methanol extract was stored at -20°C for bioassays (Abu, et al., 2017).

The crude methanol extract obtained from the above method (24 g of C-6, 54.9 g of E-6, and 20 g of B-6) was then subjected to extraction with different solvents such as hexane, ethyl acetate, and butanol (twice each) and the filtrate was concentrated under reduced pressure using a rotary vacuum. The hexane fraction (5 g of C-1, 3.0 g of E-1, 2.0 g of B-1), ethyl acetate fraction (4.7 g of C-2, 5 g of E-2, 2.7 g of B-2), butanol fraction (2.3 g of C-4, 8 g of E-4, 1.8 g of B-4), and dried aqueous fraction (8 g of C-5, 13 g of E-5, 3.6 g of B-5) were obtained (Ali, et al., 2013; Abu, et al., 2017; Hossain, et al., 2014). The active constituents of the extracts and their fractions were then analyzed by TLC. The extraction process is showed in flow chart given below (Fig. 1A, 1B and 1C).

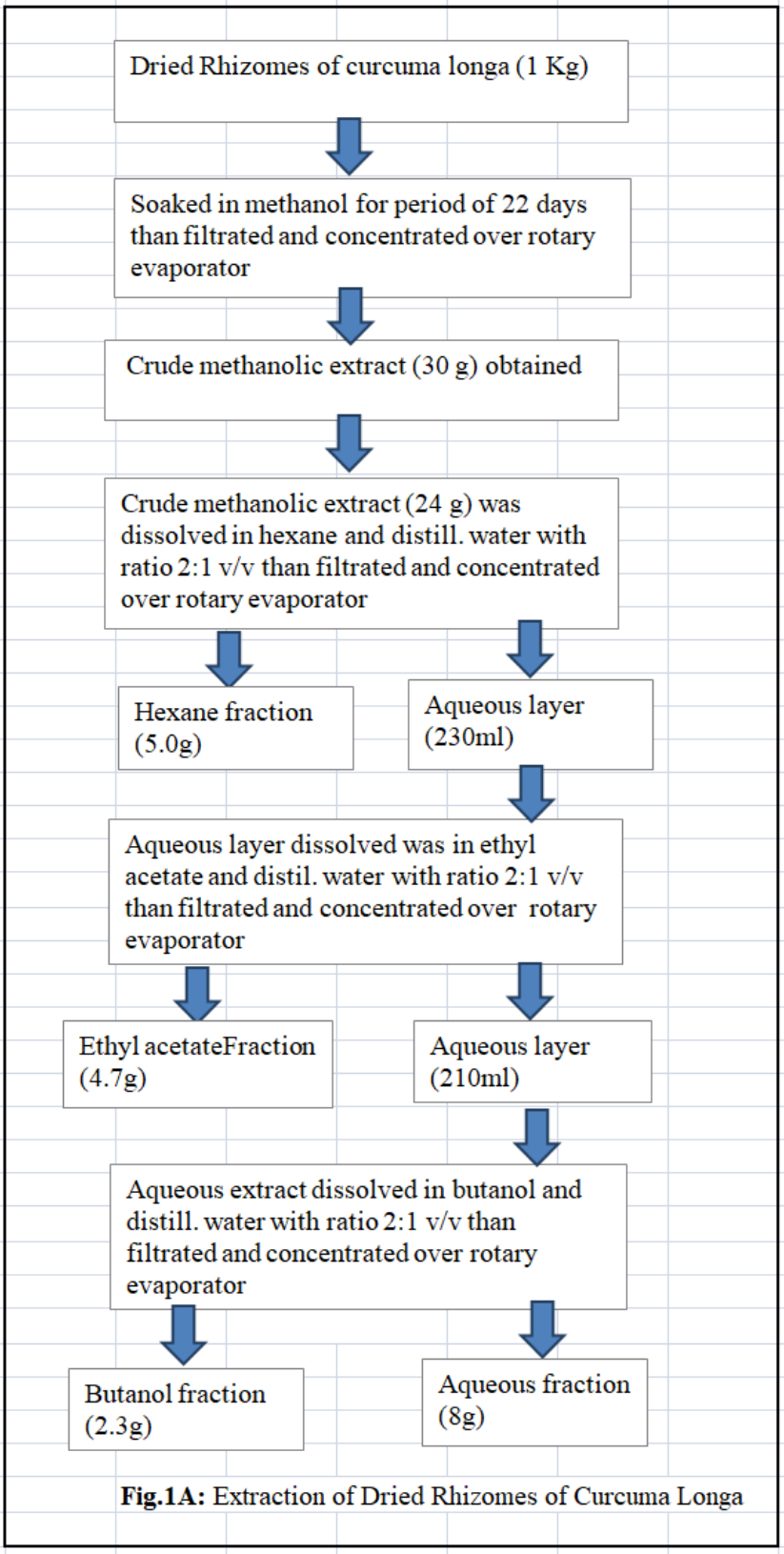

Fig.1A: Extraction of Dried Rhizomes of Curcuma Longa

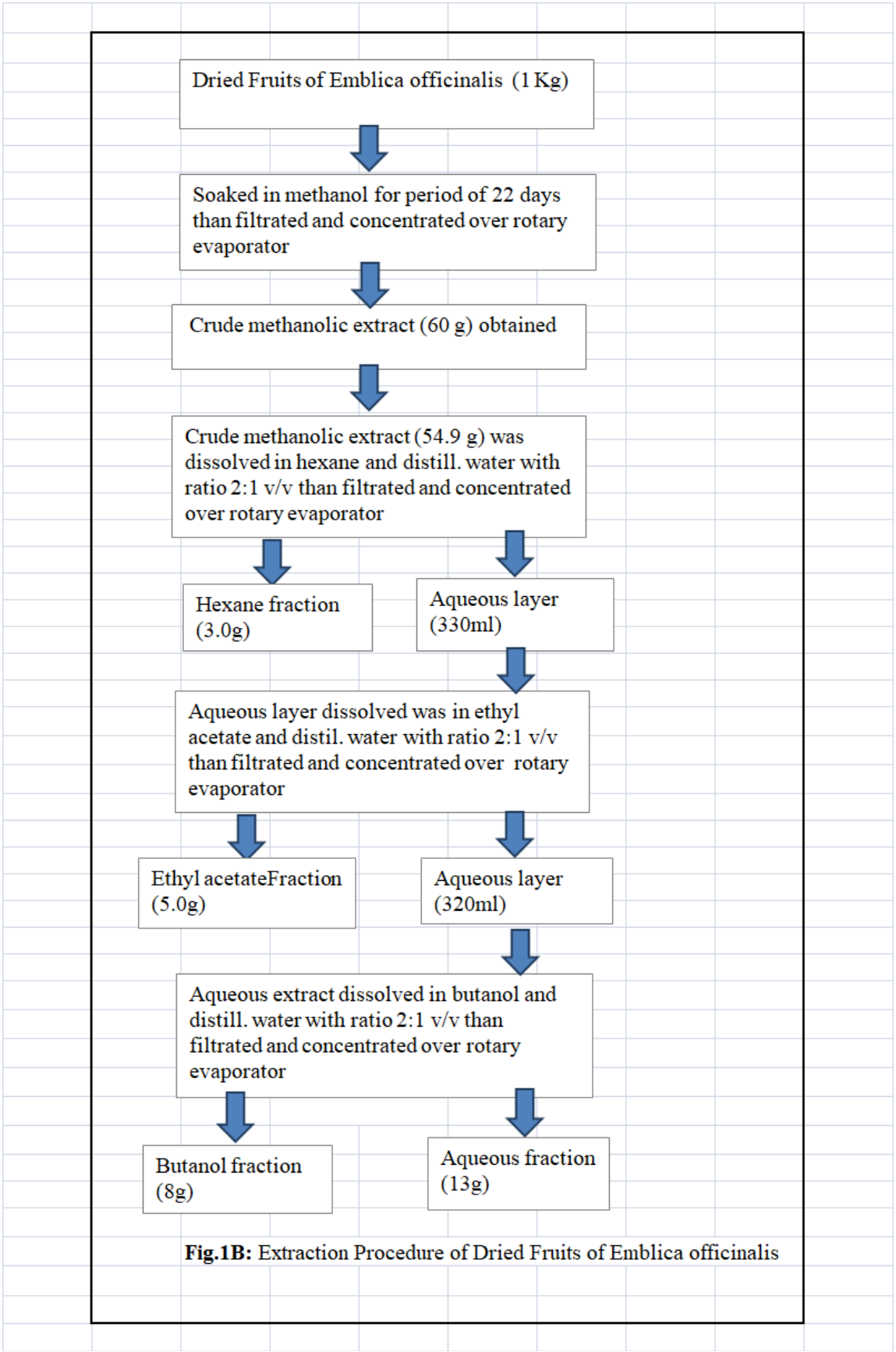

Fig.1B: Extraction Procedure of Dried Fruits of Emblica officinalis

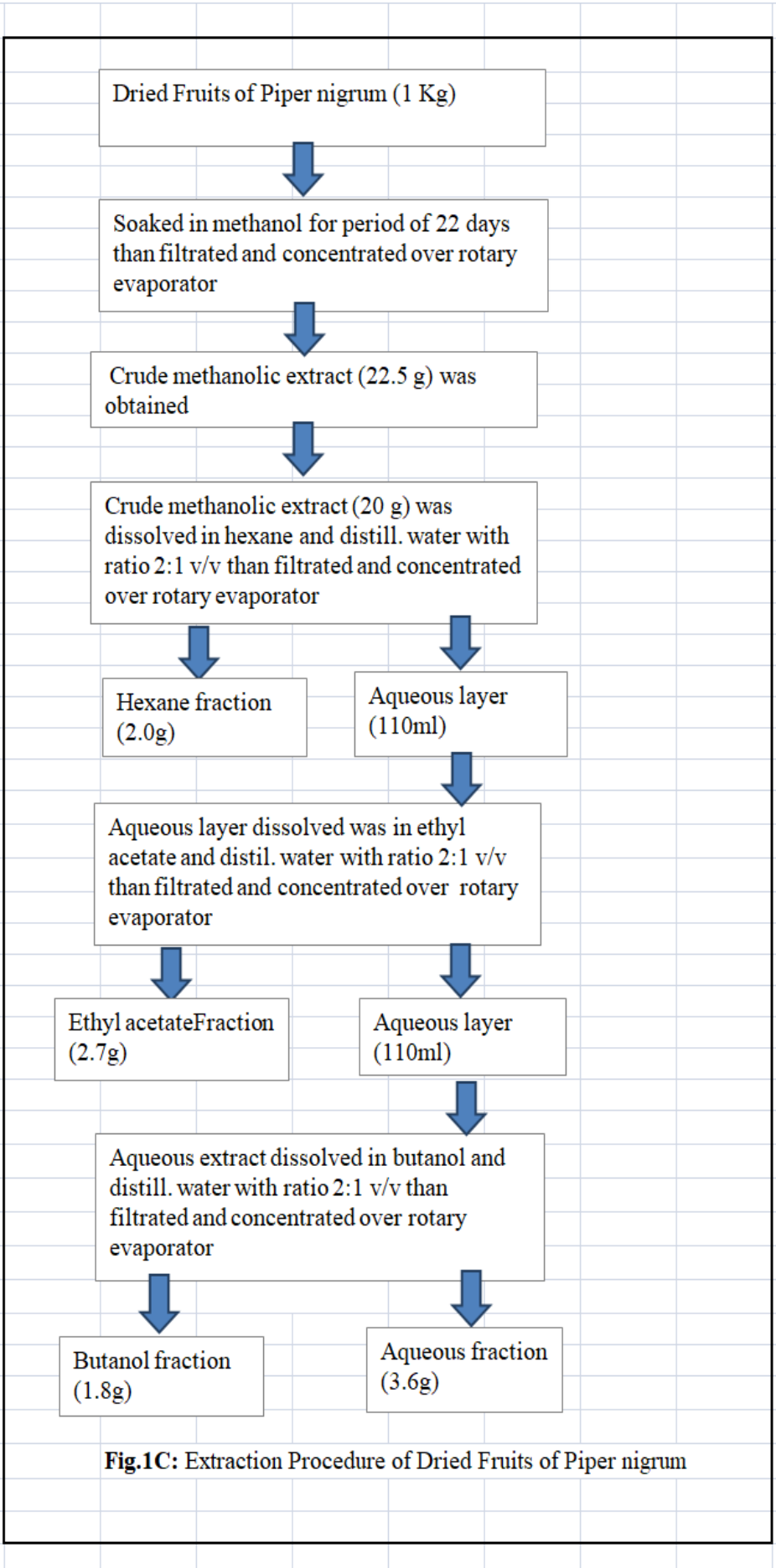

Fig.1C: Extraction Procedure of Dried Fruits of Piper nigrum

2.4. Determination of chemical components by Thin Layer Chromatography (TLC)

The crude methanolic extract and its fractions (1 mg) of three selected plants were dissolved in a solvent mixture of chloroform and methanol in a ratio of 1:1 (i.e. 1 ml each). The solutions were agitated and applied onto a stationary phase consisting of Aluminum plates (10 × 10 cm) coated with silica gel 60 F254.The plates containing the samples were developed in a TLC tank containing various solvent systems such as hexane, chloroform, ethyl acetate, and methanol. After development, the plates were visualized under UV-light (254-366 nm), and then the separated spots were marked and visualized by colorization with iodine vapours (Santana, and Meireles, et al., 2016).

2.5. Evaluation of plant extracts on insulin fibrillation using ThT Fluorescence Analysis

Human insulin fibrillation was assessed using the ThT Fluorescence method as previously described by Alam, et al. (2017) with slight modifications. Briefly a stock solution of recombinant human insulin (rHI) at a concentration of 100 µM was prepared by dissolving insulin powder in 50 mM KCl-HCl buffer, pH 1.6. Fibrils were formed by incubating insulin for 6 hours at 65°C.A stock solution of ThT was prepared by dissolving ThT in Milli-Q water from Millipore, USA and filtering it through a 0.22-micrometer filter. ThT Fluorescence Analysis was conducted using a Spectromax-5 Spectrofluorimeter from Molecular Devices, USA.

In the insulin fibrillation assay, rHI samples were incubated without or with various concentrations of plant extracts and their fractions. The samples were incubated at 65°C for 6 hours. Since the plant extracts and their fractions were not soluble in water, they were initially dissolved in DMSO and then serially diluted to achieve final concentrations of 0.19, 0.39, 0.78, 50, and 100 µg/mL. For each set, samples were taken at fixed time intervals and mixed with ThT to achieve a final concentration of 20 µM. All samples were placed in a 96-well standard plate (Nunc^{TM}, Germany), incubated for 30 minutes in the dark, and excited at 440 nm. Fluorescence emission spectra from 460 to 600 nm were recorded using the Spectromax-5 Spectrofluorimeter (Molecular Devices, USA).

IC_{50} values were determined using the trial version of Ez.fit software's. Thioflavin T Fluorescence analysis method was used to identify the development of amyloid fibrils (Siddiqi, et al., 2018).

2.6. Statistical Analysis

Statistical analysis was conducted by comparing the means of three groups using one-way analysis of variance (ANOVA). The significance of the results was determined by the p-value, with a threshold set at less than 0.05.

3. RESULTS

3.1 TLC of plants' crude methanolic extract and its fractions

Crude methanolic extracts of *Curcuma longa*(C) , *Embelica Officinalis* (E) , *Piper nigrum* (B) and their hexane (H), ethyl acetate (EA), butanolic (B), and aqueous fraction (A) were used for phytochemical profile & to evaluate anti insulin fibrillation potential. The solvent system for TLC was performed by utilizing various solvents. Later on TLC plates were developed and spots were marked with UV & Iodine vapours, in Hexane as shown in Fig 2, Chloroform in Fig. 3, Ethylacetate in Fig. 4 & Methanol in Fig. 5 (Santana, and Meireles, et al., 2016).

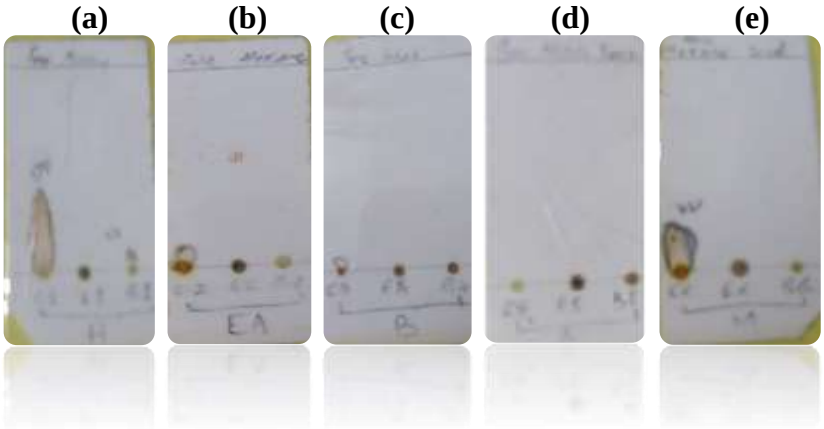

Fig. 2: TLC analysis in Hexane (a), hexane fraction of all plants (b), ethyl acetate fraction of all plants (c) butanol fraction of all plants (d).aqueous fraction of all plants (e) methanolic extracts of all plants

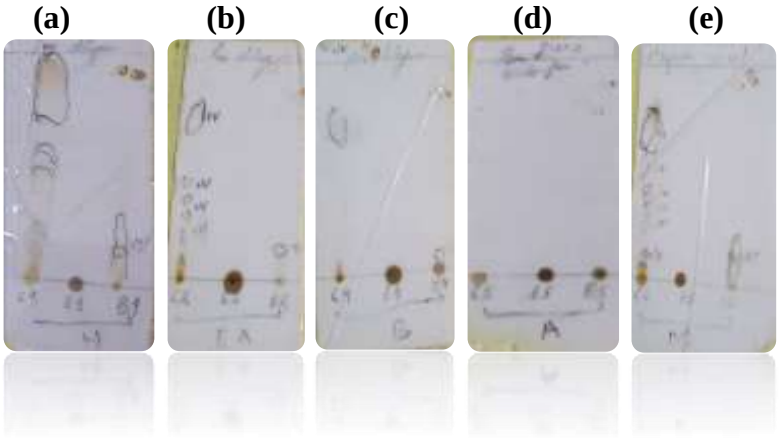

Fig. 3: TLC analysis in Chloroform (a), hexane fraction of all plants (b), ethyl acetate fraction of all plants (c) butanol fraction of all plants (d).aqueous fraction of all plants (e) methanolic extracts of all plants

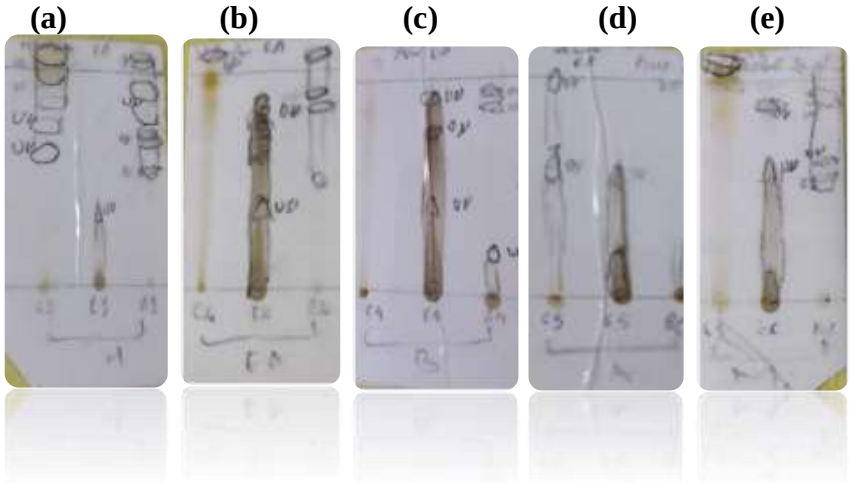

Fig. 4: TLC analysis in Ethylacetate (a), hexane fraction of all plants (b), ethyl acetate fraction of all plants (c) butanol fraction of all plants (d).aqueous fraction of all plants (e) methanolic extracts of all plants

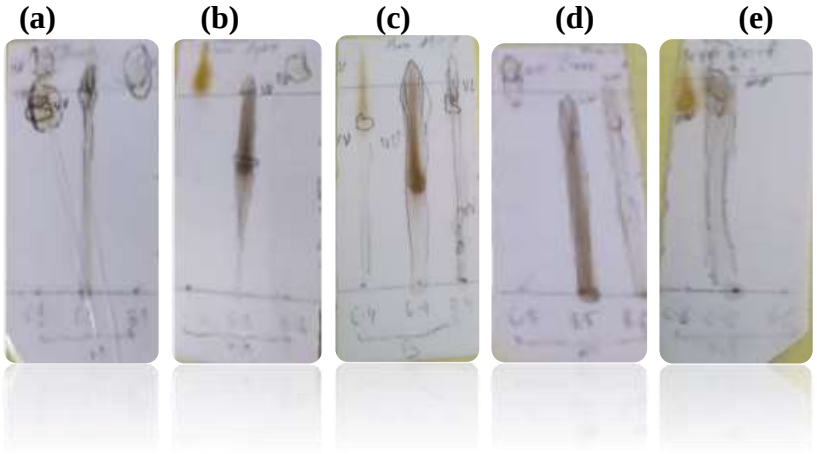

Fig. 5: TLC analysis in Methanol (a), hexane fraction of all plants (b), ethyl acetate fraction of all plants (c) butanol fraction of all plants (d).aqueous fraction of all plants (e) methanolic extracts of all plants

1.1.1. TLC of crude methanol extracts of *Curcuma longa*

TLC showed that the crude methanolic extract of *Curcuma longa* (C6) contains more chemical constituents compared to the extracts and fractions of the other plants used in this study. The TLC analysis of the crude methanolic extracts of *Curcuma longa* (C6) was performed in various solvents, including hexane, chloroform, ethyl acetate, and methanol as shown in Fig. 6.

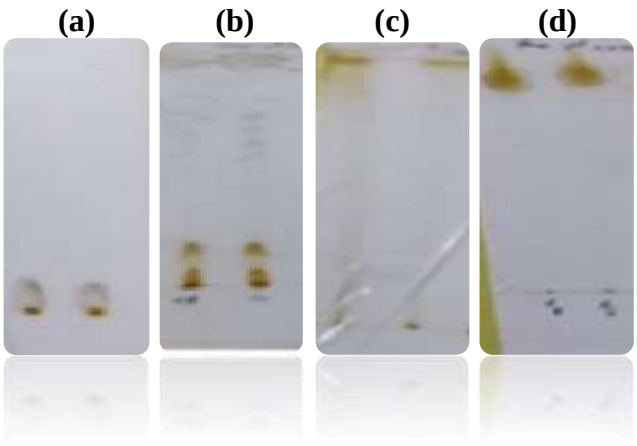

Fig. 6: TLC analysis of the methanolic extracts of *Curcuma long* in Hexane (a), Chloroform (b), Ethyl acetate (c) and, Methanol (d).

The TLC analysis of the crude methanolic extract and its fractions from *Curcuma longa* (C), *Embelica officinalis* (E), and *Piper nigrum* (B) was performed in different solvents. The solvent systems used, were hexane (100%), chloroform (100%), ethyl acetate (100%), and methanol (100%). After developing the TLC plates, they were observed under UV light and iodine vapours.

- In the hexane solvent system, only the crude methanolic extract and its hexane and ethyl acetate fractions of *Curcuma longa* showed spots of compounds (Fig. 2).
- In the chloroform solvent system, the crude methanolic extracts and their fractions of *Curcuma longa* and *Piper nigrum* displayed spots of components, while Embelica officinalis did not show any spots of active components (Fig. 3).
- In the ethyl acetate solvent systems, all the crude methanolic extracts and their fractions from the three selected plants showed spots of active components.

- In the methanol solvent systems all the crude methanolic extracts and their fractions from the three selected plants showed spots of active components except for the crude methanol extract of *Piper nigrum* (Fig. 4 and 5).

3.2. Plant extracts & its fraction; their effects on insulin fibrillation

The effect of the plant crude methanolic extracts and their fractions on insulin fibrillation was determined by ThT fluorescence measurement using 96-well standard plates. The samples were incubated without or with various concentrations of the plant extracts and their fractions. ThT fluorescence was measured at an excitation wavelength of 483 nm and an emission wavelength using the Spectromax-5 Spectrofluorimeter. The IC_{50} values of insulin fibrillation, both with and without the plant extracts, were calculated and are summarizes in Table 1 and graphically illustrated in Fig. 7.

Table 1: IC50 values of plants' extracts or its fractions

S.No.	Plants	Extracts or fractions	IC_{50} Concentration (µg/mL)
1.	*Curcuma longa*	Crude methanolic extract	**0.55**
		Hexane fraction	37.013
		Ethyl acetate fraction	41.5228
		Butanol fraction	4.7459
		Aqueous fraction	2.76
2.	*Embelica officinalis*	Crude methanolic extract	8.8662
		Hexane fraction	29.2424
		Ethyl acetate fraction	7.0828
		Butanol fraction	8.3027
		Aqueous fraction	7.6669
3.	*Piper nigrum*	Crude methanolic extract	14.9997
		Hexane fraction	32.68
		Ethyl acetate fraction	13.0472
		Butanol fraction	4.46
		Aqueous fraction	4.7459

According to Moga, et al. (2021), the effectiveness of extracts based on their IC_{50} values is categorized as follows: extracts are considered **more effective** if the IC_{50} value is less than 10 µg/ml; **effective** if the IC_{50} value is between 10 and 100 µg/ml; **mildly effective** if the IC_{50} value ranges from 100 to 500 µg/ml; and **less effective** if the IC_{50} value is greater than 500 µg/ml.

The eight plant extracts and their fractions demonstrated significant anti-insulin fibrillation activity, as evidenced by their IC_{50} values being ≤ 10 µg/ml (Fig. 7). The results showed that the amyloid fibril structure was most substantially impaired by the crude methanolic extract of *Curcuma longa*, which had an IC_{50} value of 0.55 µg/ml. This was followed by the aqueous fraction of *Curcuma longa* with an IC_{50} of 2.76 µg/ml, the butanol fraction of *Piper nigrum* with an IC_{50} of 4.46 µg/ml, the butanol fraction of *Curcuma longa* and the aqueous fraction of *Piper nigrum*, both having an IC_{50} of 4.74 µg/ml. Further, the ethyl acetate fraction of *Embelica officinalis* had an IC_{50} of 7.08 µg/ml, the aqueous fraction of *Embelica officinalis* had an IC_{50} of 7.66 µg/ml, and the crude methanolic extract of *Embelica officinalis* had an IC_{50} of 8.86 µg/ml. Among these, the highest anti-insulin fibrillation activity was observed in the crude methanol extract of *Curcuma longa* **(**IC_{50} = 0.55 µg/ml) compared to the other plant extracts and their fractions. The ThT fluorescence assay successfully illustrated that the crude methanolic extract of *Curcuma longa* significantly reduced fibril formation (Fig. 7).

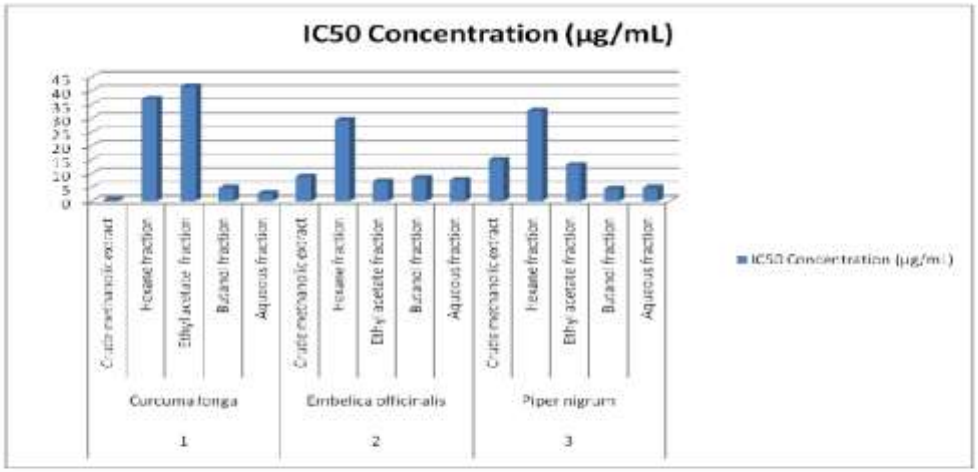

Fig. 7: Graph showing IC_{50} values of plants crude methanolic extracts and its fractions

Changes in the fluorescence of insulin indicate significant insights into its structural integrity. During fibril formation, structural deterioration lead to increased fluorescence.The variation in the fluorescence of the insulin fibril in the presence or absence of plant extracts, helps to determine the effect of these extracts and their fraction on fibril formation.Insulin fibrils not treated with plant extracts exhibited the highest fluorescence, indicating significant conformational changes during the transition to fibrils.

The effect of the crude methanolic extract of *Curcuma longa* on insulin fibril formation is shown in Fig. 8. The fluorescence intensity of ThT, measured at 483 nm, was analyzed both with and without varying concentrations of the plant extracts and their fractions (ranging from 0.19 to 100 µg). Specifically, the concentrations of the plant extracts used were 0.19, 0.39, 0.78, 50, and 100 µg. The fluorescence intensity of ThT for insulin incubated without any plant extract exhibited the highest fluorescence peak, indicating that the ThT fluorescence intensity increases when insulin fibrils interact with Thioflavin T in the absence of plant extracts. Conversely, the fluorescence intensity significantly reduced in the presence of the crude methanolic extract of *Curcuma longa*, showing a more substantial reduction in comparison to other plant extracts and their fractions. This indicates that the crude methanolic extract of *Curcuma longa* effectively binds to insulin fibrils and diminishes their fluorescence intensity, highlighting its potential in inhibiting insulin fibril formation and help in.preserving the native structure of insulin.(Fig. 8A)

In the crude methanolic extract of *Curcuma longa*, the ThT fluorescence intensity began to decrease as the concentration of the plant extracts increased, particularly within the range of 0.78 to 100 µg. This range of concentrations proved to be the most effective for suppressing fibril formation (Fig. 8B). Although all plant extracts and their fractions reduced insulin fibrillation to some extent, their inhibitory effects varied. The results suggest that the crude methanol extract of *Curcuma longa* most effectively decreases amyloid insulin aggregation compared to the other plant extracts tested.

The relationship between the log concentration of insulin fibrils with or without plant extracts and the methanolic extracts formed dose-response curves, from which IC_{50} values were derived (Fig. 8C). The goodness of fit for IC_{50} determination is indicated by the R^2 value. An R^2 value approaching 1 suggests a satisfactory fit and greater reliability of the IC_{50} value in a linear regression model (Nevozhay, et al., 2014). The R^2 value for the crude methanolic extract of *Curcuma longa* was 0.9648, indicating a highly satisfactory fit.

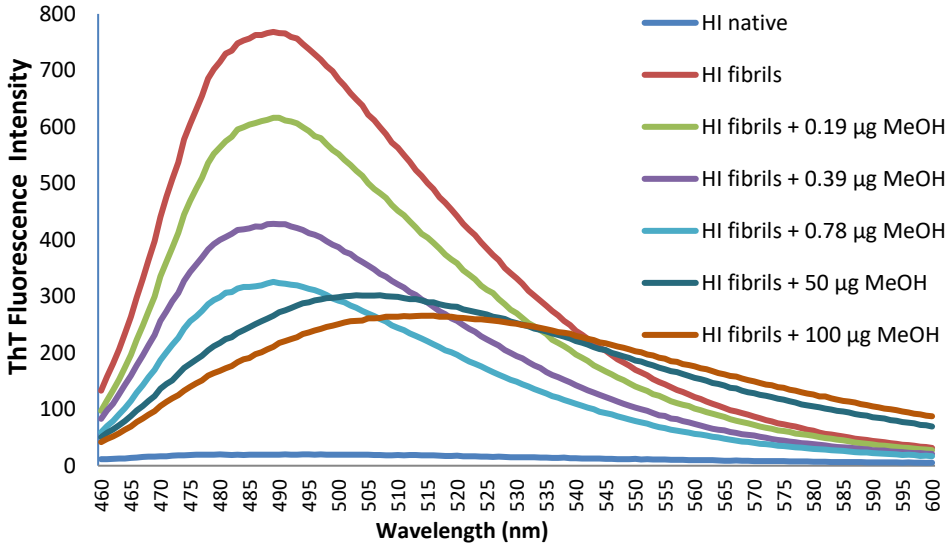

Fig. 8A: ThT assay; Insulin ThT fluorescence intensity of insulin was measured at 483nm for insulin solutions treated with varying concentrations of the crude methanolic extract of *Curcuma longa.* The conditions were as follows: : control (HI fibril without extract), HI fibril + 0.19 µg crude methanol extract, HI fibril + 0.39 µg crude methanol extract, HI fibril + 0.78 µg crude methanolic extract, HI fibril + 50 µg crude methanolic extract, and HI fibril + 100 µg crude methanolic extract. The samples were incubated for 6 hours under acidic conditions (pH 1.6)at 65°C as aforementioned in materials and methods section

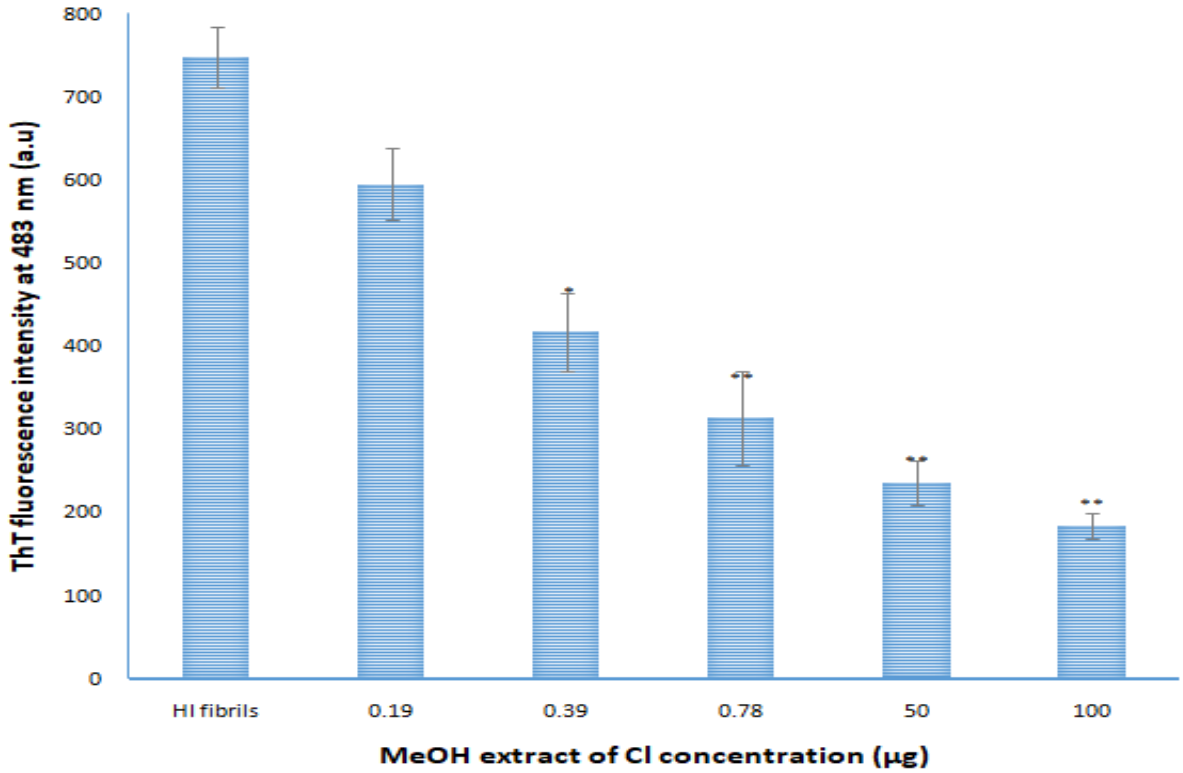

Fig. 8B: ThT analysis of insulin fibril treated with or without plant extracts under pH 1.7 subsequently incubated at 65°C for 6 hours. The conditions tested included: control (HI fibril without extract), HI fibril + 0.19 µg crude methanolic extract, HI fibril + 0.39 µg crude methanolic extract, HI fibril + 0.78 µg crude methanolic extract, HI fibril + 50 µg crude methanolic extract, and HI fibril + 100 µg crude methanolic extract. In statistic, *P < 0.05 & **P < 0.01 was correlated with insulin fibril

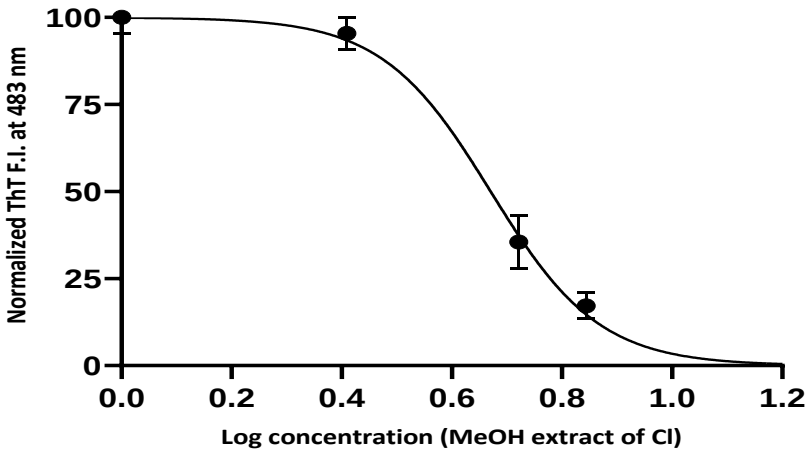

Fig. 8C: Dose-response Curve for Determining IC_{50} of Crude Methanolic Extracts of Curcuma longa Compared to Insulin Aggregation using the ThT assay. The normalized fluorescence intensity of ThT at 483nm was plotted against the logarithm of the concentration of crude methanolic extract of Curcuma longa

DISCUSSION

Herbal drugs are gaining more attention worldwide due to having fewer adverse effects compared to synthetic drugs. Remedial plants serve as a source of bioactive compounds that are naturally derived (Momenkiaei, F., and Raofie, F., 2019).

Amyloid fibrils are protein aggregates related with Alzheimer's diseases that are self-built, (Ow, and Dunstan, 2014).Still no appropriate treatment of Alzheimer is attainable so Alzheimer disease are remediless. In worldwide, conventional medicine accomplished as a memory booster from old times (Akram, and Nawaz, 2017). Amyloid fibrils are main feature of Alzheimer's diseases and suppression of Aβ fibril origination are interesting remedial scenario for Alzheimer treatment (Mishra, and Palanivelu, 2008). The goal of the study was to investigate the anti-Alzheimer potential of Crude methanolic extracts of three plants i.e. *Curcuma longa*, *Embelica Officinalis*, *Piper nigrum* and their hexane, ethyl acetate, butanolic, and aqueous fractions by insulin fibrillation prohibition

Insulin is a natural peptide harmone used in Diabeties Mellitus theraphy,Approaches developed for anti insulin fibrillation activity, act as representative in Amyloid study(Das et al., 2022).

The outcomes from this research indicate that Crude methanolic extract of Curcuma longa has highest chemical components as compared to other two plant crude methanolic extract and their fractions. ThT fluorescence method used in this study indicated that crude methanolic extract of Curcuma longa has highest insulin aggregation/fibrillation activity having [**IC_{50} 0.55 µg/mL**] among above plant crude methanolic extracts and their fractions (Table 1). Extracts are considered more effective if the IC_{50} value is less than 10 µg/ml (Moga, et al., 2021). The R^2 value for the crude methanolic extract of *Curcuma longa*was is 0.9648 indicating a highly satisfactory fit.R^2 value approaching 1 suggests a satisfactory fit and greater reliability of the IC_{50} value in a linear regression model (Nevozhay, et al., 2014). The R^2 value for the crude methanolic extract of *Curcuma longa*was 0.9648, indicating a highly satisfactory fit.

Previous study provide the information of successful anti-Aβ fibrillation activity of plants (Centella asiatica, Convolvulus pluricaulis, Bacopa monnieri and Withania somnifera) extracts and nutrient foods for Alzheimer treatment and indisguishable result of curcuma longa (Witter, et al., 2018). Former report showed Anti-Alzheimer activity of ethanolic extract (45%) of 3 plants mixture i.e. Chaenomeles sinensis,zingiber officinale and curcuma longa (2:1.5:1).by inhibition of Aβ plaques in mice (Kim, et al., 2019).

In this study, we present a novel comparison of the individual anti-Alzheimer activity of crude methanolic extracts and fractions of *Curcuma longa*, *Emblica officinalis*, and *Piper nigrum* by evaluating their ability to suppress insulin fibrillation. This research confirmed that Curcuma extracts.have neuroprotective action in Alzheimer's disease as reported in earlier studies present in literature (Randino, R et al., 2016).

CONCLUSION

As, aimed in this study, we compared the anti-insulin fibrillation potential of various plant extracts and their fractions. Overall, our findings propose plant extracts that bear the potential inhibit insulin fibrillation (amyloid fibrillation), the pathological process involved in Alzheimer's disease. Current research illustrated that the crude methanolic extract of *Curcuma longa* (C), compared to *Embelica officinalis* (E) and *Piper nigrum* (B), not only showed a greater number of spots on TLC plates (i.e. higher number of phytochemicals) but also showed superior anti-insulin fibrillation activity. Based on TLC analyses, the crude methanolic extracts of *Curcuma longa* (C), along with its hexane and ethyl acetate fractions, showed presence of higher levels of phytochemicals. While, according to the ThT assay, the methanolic extracts of *Curcuma longa* exhibited higher potential for anti-insulin fibrillation activity with an IC_{50} value of 0.55µg/ml and an R2 value of 0.9648.

This study identifies the potential of phytochemical compounds from plants sources responsible for anti-insulin fibrillation activity thus, opening new horizons for further research in this direction that could lead to the the development of new medications for treating Alzheimer's disease in the future.

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