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Anti-diabetic and anti-inflammatory activity of different metabolites extracted from *Mesua ferrea* using chromatographic techniques

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Abstract
Overuse of antimicrobial drugs has led to selective resistance to existing antibiotics, necessitating the development of different and improved alternatives. Natural substances, particularly those derived from plants, are well-known for their therapeutic characteristics, including antibacterial and antifungal properties. The main objective of the present study was to evaluate the anti-inflammatory activity of isolated bioactive flavonoid *Mesua ferrea* in-A from the bark of *Mesua ferrea* L. by *in vivo* approach. The Alpha Amylase Inhibition Assay was used to assess antidiabetic effectiveness *in vitro*. Fractions 3 and 5 displayed the most activity. Using reducing power, an antioxidant activity test was performed. Fraction no. 3 showed a higher absorbance of 0.94 at 500 g/ml.

Keywords: Chromatography, *Mesua ferrea* Linn, anti-diabetic and anti-inflammatory activity, secondary metabolites

Introduction
Mesua ferrea Linn is a medium to big evergreen tree of the Clusiaceae family that is extensively spread in India, Burma, Nepal, Thailand, Indochina, and New Guinea (Van Sam H. *et al*. 2004, Byrne, C., 2018) [119, 21]. It is found in the lower Himalayas from Nepal, Bengal, Andhra Pradesh and the Andaman and Nicobar Islands in India (Nadpara, N. 2012, Barbade, K. D., &Datar, A. G. 2015, Shome, U.1982, Chitte, R. 2016) [82, 18, 105, 28] and reach elevations of up to 1500 metres (Siram, O., 2022, Rajilesh, V. K. 2019) [105, 93]. The plant is widely used in traditional medicine for a variety of pharmacological activities and is used in a variety of traditional medicinal preparations such as Nagkeshara-adi-churna, Nagkeshara Yoga, Eladi Churna, Lavangadi Churna, and Dasamoolarishta, among others (Barbade, K. D., & Datar, A. G. 2015) [18]. The seeds, leaves, and stem bark of *Mesua ferrea* have been researched for a variety of therapeutic qualities including antioxidant and antibacterial activity, analgesic, antispasmodic, and anti-venom action, immunomodulatory and anti-arthritic potentials, and anti-venom activity (Chahar M. *et al*. 2013, Kotteswari M, *et al*. 2018, Sharma, A., *et al*. 2017) [22, 63, 102]. The flowers of the plant have also been researched for their therapeutic including immunomodulatory properties (Chakraborty, D. *et al*. 2023, Subramaniam, K., *et al*. 2021 Asif, M., *et al*. 2017, Rouger, C., *et al*. 2019, Kshirsagar, P. R., &Patil, S. M. 2020, Zhang, D., *et al*. 2021, and Gupta, A., *et al*. 2014) [23. 114, 11, 97, 65, 125, 47], anti-diabetic (Balekari, U., & Veeresham, C. 2015, Hasan, M., *et al*. 2020, Gupta, A., &Chaphalkar, S. R. 2015) [17, 51, 46], anti-inflammatory (Rajalakshmi P. *et al*. 2019, Gupta, A., & Chaphalkar, S. R. 2015, Murthuza, S., & Manjunatha, B. K. 2018, Nakyai, W., *et al*. 2021) [92, 46, 81, 83]. Its flower's volatile oil displayed antibacterial, antifungal, and anthelmintic properties (Keawsa-Ard, S., *et al*. 2015, Rmw, L., *et al*. 2020, Joseph, C. *et al*. 2010, Drissi, B. *et al*. 2022, Tadesse, S. *et al*. 2011) [58, 96, 57, 40, 115]. The dried blossoms of the plant are scented and used to cure bleeding piles, diarrhoea, cough, and as a carminative (Shubhashree, M. *et al*. 2015, Kumari, R., 2019, Selvam, A. 2008, Maneesha S. *et al*. 2021, and B Aggarwal, *et al*. 2011) [106, 67, 99, 77, 5, 16]. Furthermore, this *Mesua ferrea* plant products demonstrated antibacterial action due to proteins or peptides (low molecular mass) that were tested (Nakyai, W., *et al*. 2021, Khameneh, B., *et al*. 2019, Zaid, A. N., & Al Ramahi, R. 2019, Seukep, A. J., *et al*. 2020,

Vaou, N., *et al*. 2021) [83, 59, 124, 100, 120]. *In-vivo* and *in-vitro* antioxidant activity of methanol and ethanol extracts of *Mesua ferrea* flowers has also been documented (Barbade, K. D., & Datar, A. G. 2015, Chitte, R. *et al*. 2016, Plekratoke K., *et al*. 2023) [18, 28, 90]. However, only a little amount of study has been recorded for the stamens of *Mesua ferrea*, which constitute a prominent element of *Mesua ferrea* flowers because to their abundance. However, there is a scarcity of scientific evidence on their therapeutic potentials, phytochemical analyses, and *in-vivo* safety (Khanduri, V. 2023, Shelke, R. G., & Rangan, L. 2022, Raman, T. 1998, Krishnadas, M., Chandrasekhara, K., & Kumar, A. 2011) [61, 104, 94, 64].

New synthetic antimicrobial, antioxidant, antidiabetic, and anti-inflammatory drugs have been developed as a result of the increased prevalence of multiple drug resistance (Govindappa, M. *et al*. 2011, Confederat, L. G., *et al*. 2021, Hamidpour, R., *et al*. 2017) [43, 30, 50]. In addition, the new drug is required to look for new antimicrobial, antioxidant, antidiabetic, and anti-inflammatory from alternative sources (Martelli, G., & Giacomini, D. 2018) [78]. Bioactive compounds from medicinal plants with pharmacological activities have the ability to meet this demand since their structures diverge from those of the most explored plants, although those with more activity may differ (Atanasov, A. G., *et al*. 2015, Cucu, Alexandra-Antonia, *et al*. 2022, Gracz-Bernaciak, *et al*. 2021) [13, 31, 44]. The fast rise of diverse drug-resistant pathogen strains to present antimicrobial medicines has created an urgent need for novel antibiotics derived from medicinal plants (Amenu, D. 2014, Vaou, Natalia, *et al*. 2021, Cheesman, Matthew J., *et al*. 2017, Subramani, R., *et al*. 2017, Abdallah, E. *Et al*. 2011, Anand, U., *et al*. 2019) [9, 120, 26, 113, 1, 10]. Many medicinal plants have been intensively tested for antibacterial capabilities all throughout the world (Das, K. *et al*. 2010, Mahady, G. B. 2005, Atanassova, M. *et al*. 2011, Farzaneh, V., & Carvalho, I. S. 2015, Debnath, M. 2008) [32, 74, 14, 41, 35]. In normal or pathological cell metabolism, free radicals with one or more unpaired electrons (superoxide, hydroxyl, peroxyl) are formed, and substances that can scavenge free radicals have a high potential for treating illnesses and sick cells (Young, I. S., & Woodside, J. V. 2001, Devasagayam, T. *et al*. 2004, Agarwal, A., *et al*. 2006, Singh, R. *et al*. 2004, Khan, F., *et al*. 2018, Valko, M. *et al*. 2007) [123, 37, 2, 108, 60, 118]. Thus, antioxidants play a crucial role in protecting the human body from harm caused by reactive oxygen species. Diabetes is a chronic carbohydrate, lipid, and protein metabolic condition characterised by elevated fasting and postprandial blood sugar levels (Lozano, I., *et al*. 2016, De Silva, *et al*. 2012, Singh, A. *et al*. 2021, Avignon, A., 2012, Amalan, V., 2016, Shali, K. 2022) [73, 33, 107, 15, 7]. Inflammation is a physical reaction to damage, infection, or destruction that is characterised by heat, redness, discomfort, swelling, and disrupted physiological activities. The release of chemical mediators from wounded tissue and migratory cells causes it to occur (Divya, R. *et al*. 2016, Chandra, S. *et al*. 2012, Tiwari, Y. *et al*. 2021, Gunalan, S., *et al*. 2020, Chatterjee, P. *et al*. 2012, Asija, R., *et al*. 2014) [39, 24, 117, 45, 25, 12]. The *Mesua ferrea* Linn. proved beneficial for several system illnesses, and it is the third most used medicine by domestic industries in terms of volume, behind Amalaki, and Hareetaki (Sharma, R., *et al*. 2019, Devi, Y. D. (2012) [103, 38]. *Mesua ferrea* Linn. is a member of the Clusiaceae (Syn. Guttiferae) family. It consist two new bioflavonoids and lupeol type of tri-terpenoid (Saxena, H. *et al*. 2022, Kumar S. 2014) [98] which are beneficial to treat various disorders like Diarrhoea, internal haemorrhages, menorrhagia, scabies, skin eruptions, itching, small tumours, headache, blood and heart troubles, sore throat, cough, hiccough, vomiting, thirst, dysentery, and bleeding piles (DeFilipps RA, and Krupnick GA. 2018, Aggarwal B. *et al*. 2011, Khare, C. P., 2008) [36, 5, 16]. Its bark is an excellent demulsifier (Lemos, R. *et al*. 2010) [71], its fresh blooms are aromatic, bitter, and stomachic; and its leaves are aromatic, bitter, and stomachic dried blossoms are stimulant and carminative, its unripe fruits are aromatic, acrid and purgative (Selvam, A. 2008, Lata, S. 2019) [99, 69]. In this research, we have used the bioactive compounds from *Mesua ferrea* Linn. to perform ant-diabetic and anti-inflammatory activity.

Materials and Methods

Column chromatography, TLC and Lyophilisation

Column chromatography

In column chromatography, the column 2 cm x 25 cm was packed with a solution of silica gel with water using the wet slurry method (Patra, J. K., 2012, Patil, A. M., *et al*. 2023, Patra, J. K., & Thatoi, H. 2013, Minai-Tehrani, D., & Herfatmanesh, A. 2007) [88-89, 85 86-87, 80]. A ball of wool (glass wool) was pushed into the column to settle atop the packed silica gel (Mandal, S., 2017, Milton, G. M., & Brown, R. M. 1993) [76, 79]. The solvent system of n-butanol: acetic acid: water (4:1:1) was poured continuously into the column and allowed to drained and about 8 fraction of 5 -6 ml was collected in sterile centrifuge tube (Hu C., 2023, Chitte, R. R., *et al*. 2016) [53, 28]. The fraction eluted on column was tested with same solvent system by TLC for the presence of active compounds (Patra J. *et al*. 2012, Patra, J. K., & Thatoi, H. 2013) [88-89, 86-87].

Thin layer chromatography (TLC)

Fraction eluted on column was subjected to TLC as per conventional one dimensional ascending method using silica gel ($60F_{254}$ MERCK) pre-coated plate (Jain M.*et al*. 2010, Mallavadhani, U. V. *et al*. 2019, Takale, N., *et al*. 2023) [54-55, 75, 116]. For TLC applied sample volume 1µl by using capillary and solvent system was used is *n*- butanol: acetic acid: water. After pre-saturation with mobile phase 20 min for development of band were used (Agatonovic-Kustrin, S., 2023, Amarasiri, S. S., 2023, Akram, M. N., 2021, Nöst, X., 2021 Wianowska, D., & Olszowy-Tomczyk, M. 2023) [4, 8, 6, 84, 122]. After run the plates they are dried using dryer and plates were observed under various wavelength at 254nm and 366nm for band detection (Lawag, I. L., 2022, Hakim, M., & Patel, I. 2022, Agatonovic-Kustrin, S., 2020) [70, 49, 3]. Colour of the spot and pattern were observed and RF value were calculated using formula:

$$\text{RF (Retention factor)} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Lyophilisation

Lyophilisation was done for prolonged storage of sample that will no longer allow biological growth or chemical reaction in this process (Wang, W. 2000, Gaidhani, K. A., 2015) [21, 42]. The 1 ml column eluted pure fraction of bioactive compound was lyophilised using lyophilizer for 4 to 5 hours to get the complete dried powder (Smith, M. A. L., 2000, De Zoysa, M., 2008, Previtera, L., 2016, Jayaprakasha, G. K., 2007, Hayashi, T., 1996) [110, 34, 91, 56, 52]. The powder retested for *In vitro* activity and store at 4 °C.

Anti-diabetic activity

Antidiabetic activity of *Mesua ferrea* Linn was done by following methods

Inhibition of alpha amylase enzyme Standard maltose curve Alpha amylase inhibition assay.

Amount of maltose produced is calculated using standard maltose curve and enzyme activity is calculated by using formula

$$\text{Enzyme activity} = \frac{\text{Amount of maltose formed x 2}}{\text{10 X 342}}$$

Anti-inflammatory Activity:

Anti-inflammatory activity of *Mesua ferrea* Linn was done by using Albumin Denaturation Assay and the percent inhibition of protein denaturation was calculated as follow;

$$\text{Percentage inhibition} = \frac{\text{Abs. control - Abs. sample}}{\text{Abs. control}} \text{ x 100}$$

Membrane stabilization test

Preparation of red blood cells (RBCs) Suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tube. The tubes were centrifuged at 3000 rpm for 10 min. and were washed three times with equal volume of normal saline. The volume of blood was measured and constituted as 10% v/v suspension with normal saline (Sakat *et al*., 2010) [126].

Heat Induced Haemolytic:

The reaction mixture 2 ml consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension**.** Instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug.

All the centrifuged tube containing reaction mixture was incubated in water bath at 56 °C for 30 min.At the end of the incubation the tube were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min. and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicate for all the test sample, % membrane stabilization activity was calculated by formula; (Shinde *et al*., 1999 and Sakat *et al*., 2010) [127, 126]

$$\text{Percentage inhibition} = \frac{\text{Abs. control - Abs. sample}}{\text{Abs. control}} \text{ x 100}$$

Protein Inhibitory Action

The test was performed according to the modified method of Oyedepo *et al*., (1995) [128] and Sakat *et al*., (2010) [126]. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 Mm Tris HCL buffer (pH 7.4) and 1 ml test sample of different concentration. The reaction mixture was incubated at 37 °C for 5 min. and then 1 ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min., 2 ml of 70% Perchloric acid was added to terminate the reaction. Cloudy suspension was read at 210 nm against buffer as blank. The experiment was performed in triplicate (Leelaprakash G., Dass. Mohan 2011) [129] Percentage protein inhibition activity was calculated by formula;

$$\text{Percentage inhibition} = \frac{\text{Abs. control - Abs. Sample}}{\text{Abs. control}} \text{ x 100}$$

Table 1: Phytochemical analysis of *Mesua ferrea*

Sr. No.	Name of phytochemicals	Test	Inference	Observation
1	Alkaloid	Add 2 ml of extract to 2N HCL decand aqueous layer formed and few drop of mayers reagent	Cream precipitate observe indicating the presence of alkaloid	Cream precipitate was observed
2	Phenolic compounds	Compounds-Add 3-5 drops of 5% $FeCl_3$ solution to 2 ml of extract	Formation of deep blue colour	Deep blue colour was observed
3	Flavonoids	In 2 ml of extract, add 2-5 drops of 1N NaOH	Formation of yellow orange colour	Yellowish orange colour seen
4	Saponins	Add 2 ml of extract with 6 ml of water in a test tube	Observe for persistent foam	Observation of persistent foam
6	Tannins	Add 2 ml of aqueous extract with 2 ml of distilled water and few drops of $Fecal_3$	Formation of green precipitate	Green precipitate was observed
7	Leucoanthocyanins	Add 5 ml of aqueous extract to 5 ml of isoamyl alcohol.	Upperlayer appears red in colour	Red colour was not observed
8	Quinone	Add 2 ml of extract with concentrated HCl	Formation of yellow precipitate	Yellow precipitate was observed
9	Coumarin	Add 3 ml of 10% NaOH to 2 ml of aqueous extract	Formation of yellow colour	Yellow colour was observed
10	Steroid	Dissolve 1 ml of extract in 10 ml of chloroform and add equal volume of concentrated H_2SO_4	The upper layer turns red and H_2SO_4 layer shows yellow green fluorescence	The upper layer turns red and H_2SO_4 layer yellow green fluorescence
11	Emodins	Add 2 ml of extract with concentrated HCl	Formation of yellow precipitate	Yellow precipitate was observed
12	Phlobatanin	Add 2 ml of aqueous extract to 2 ml of 1% HCl and boil the mixture.	Deposition of red precipitate	Red precipitate was not observed
13	Anthocyanin	Add 2 ml of aqueous extract to 2 ml of 2N HCl and Ammonia	Appearance of pink- red urns Blue-violet	Pink-red colour turns Blue-voilet

Results and Discussion

Column chromatography and TLC studies

Thin layer chromatographic studies of partial purified methanol fraction of *Mesua ferrea* Linn was done by using silica gel 60 F_{254} (MERCK) aluminium plate. Solvent system *n*-butanol: aceticacid: water (4:1:1) was used for separation of compound. Partial purified fraction eluted on column chromatography showing different band pattern at 254 nm and 366nm.spot were characterized by Rf value under UV light

Table 2: TLC investigation and banding pattern for column eluted fractions

fraction no.	Solvent system	No. of spot detected		Rf value	
		254 nm	366 nm	254 nm	366 nm
1	n-butanol: Acetic acid: water	-	-	-	-
2	n-butanol: Acetic acid: water	1		0.80	-
3	n-butanol: Acetic acid: water	1	2	0.77	0.77,0.74
4	n-butanol: Acetic acid: water	1	1	0.85	0.85
5	n-butanol: Acetic acid: water	1	2	0.87	0.87
6	n-butanol: Acetic acid: water	1	1	0.85	0.85,0.86
7	n-butanol: Acetic acid: water	-		-	
8	n-butanol: Acetic acid: water	-	-	-	-

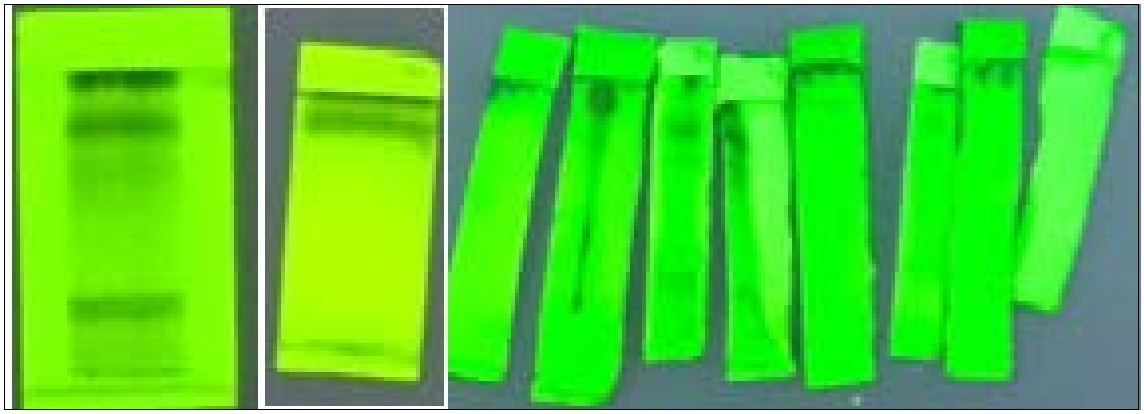

Fig 1: TLC profilling and bandling pattern of column eluted fraction at 254 nm by using n-butanol: acetic acid: water as a solvent system

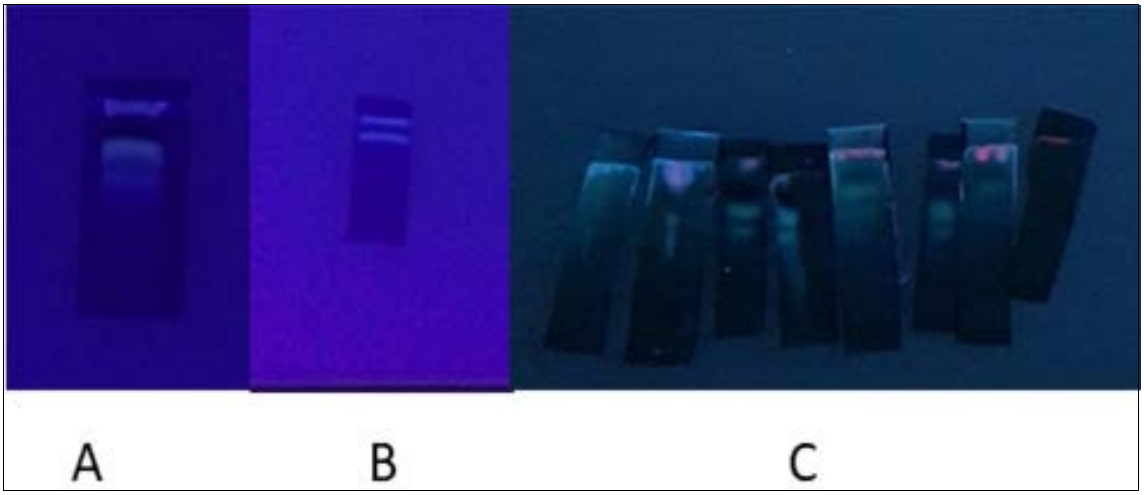

Fig 2: TLC profiling and banding pattern of column eluted fraction at 366 nm using n-butanol: acetic acid: water as a solvent system

Lyophilisation of column eluted fractions

Pure compounds were lyophilized and stored at 4 °C for better stability and long life of compounds.

Estimation of protein content of column eluted fractions using Nano Drop spectrophotometer 3.2.1

3.2.2

Table 3: Determination of Protein concentration by Nanodrop technique

Fraction no	Protein concentration
1 (water eluted)	0.650
2 (water eluted)	60.078
3 (solvent eluted)	158.222
4 (solvent eluted)	66.102
5 (solvent eluted)	25.610
6 (solvent eluted)	46.433
7 (solvent eluted)	12.303

The NanoDrop spectrophotometer from NanoDrop technologies is designed for measuring nucleic acid, protein concentration in sample volumes of one microliter. Column eluted 7 fraction of *Mesua ferrea* Linn were checked for protein determination in which fraction No. 3 showed highest158.222 protein concentration followed by fraction 4 (66.102), fraction 6(46.433)

***In vitro* Anti-inflammatory activity: Albumin denaturation assay:**

Denaturation of protein is a well-documented cause of inflammation. As a part investigation on the mechanism of the anti- inflammatory activity, ability of fraction to inhibit denaturation was studied. Column eluted fraction were effective in inhibiting albumin denaturation maximum inhibition of 84.67% was observed at 500µg/ml, aspirin a standard drug show maximum inhibition 89.23% at concentration of 500 µl/ml. Dr. Manjunatha, Divakara R. *et al*. 2013 [81] reported that the heat induced denaturation of protein was effectively inhibited by pet ether and methanol extract resp. 23.52±2% and 47.40±2%).

Table 4: Percentage inhibition of Albumin denaturation assay

Test sample	Albumin Denaturation
Fraction 3	84.67±0.010
Fraction 4	69±0.006
Fraction 6	40.89±0.010
Fraction 7	50±0.010
Aspirin	89.23±0.010

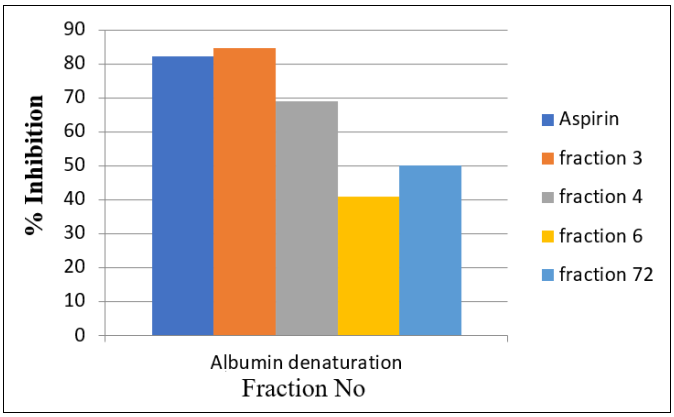

Fig 3: Percentage inhibition of Albumin denaturation assay on column eluted fractions of Nagkesar

Membrane stabilization assay

The HRBC membrane stabilization has been used as a method to study the *in vitro* anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the column eluted fraction may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosome constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. purified fraction were effective in membrane stabilization at different concentration as shown in Table, maximum inhibition of fraction no.3. 73.6% was observed at 500 µg/ml, followed by fraction 4(68%) & fraction no.6 (64%) Aspirin a standard drug show maximum inhibition 79.85% at concentration of 500 µg/ml.5. Dr. Manjunatha, Divakara R. 2013 [81] reported that the HRBC membrane stabilization effect (by inhibiting hypotonicity induced lyses of erythrocyte membrane) of methanol extract at a concentration of 500 µg/ml was 34.20±0.2% and 78.20±0.2% and standard drug Diclofenac was showed 73.00% protection.

Table 5:% inhibition of Membrane Stabilization assay

Test Sample	Membrane Stabilization assay
Fraction 3	73.6±0.010
Fraction 4	68±0.010
Fraction 6	56.60±0.010
Fraction 7	64±0.010
Aspirin	79.85±0.006

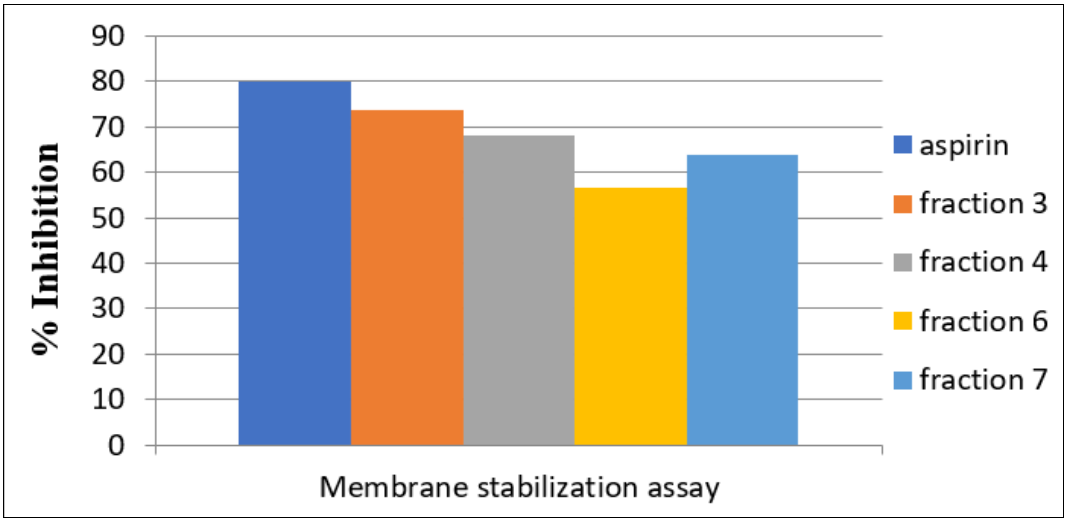

Fig 4: Membrane stabilization assay of column eluted fractions of *Mesua ferrea* Linn

Proteinase inhibitory activity

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. *Mesua ferrea* Linn partial purified fraction exhibited significant antiproteinase activity at different concentrations as shown in Table 3.6.3. Maximum inhibition of fraction no.3 90.62% was observed at 500 µg/ml, followed by fraction 7(71.25%) & fraction 4(62.5%) aspirin a standard drug show maximum inhibition 88.48% at conc.500 µg/ml. Fraction 3 has maximum inhibition and good anti-inflammatory activity. Dr. Manjunatha, Divakara R. 2013 [81] reported that proteinase inhibitor activity of pet ether and methanol extracts of *Mesua ferrea* Linn was found to be 40.66±0.2% and 50.73±0.2% respectively at the concentration of 500 µg/ml of plant extract.)

Table 6: Percentage inhibition of Proteinase denaturation of column eluted fractions of *Mesua ferrea* Linn

Test sample	Proteinase inhibition
Fraction 3	90.62±0.032
Fraction 4	62.5±0.008
Fraction 6	55±0.008
Fraction 7	71.25±0.008
Aspirin	88.48±0.007

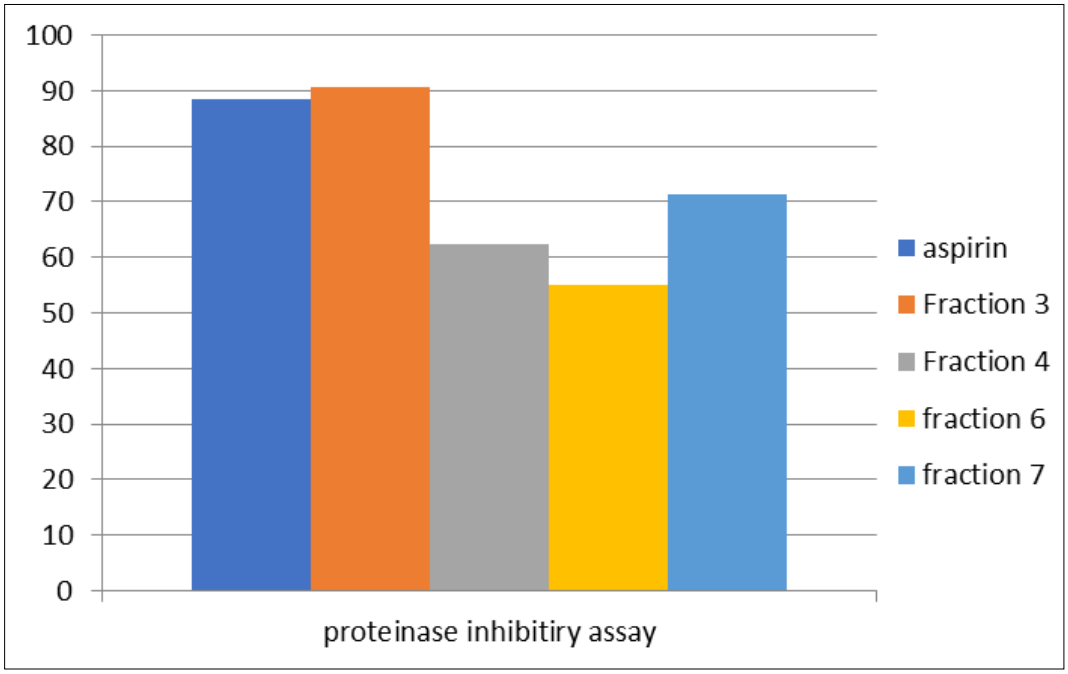

Fig 5: Proteinase denaturation of column eluted fractions of *Mesua ferrea* Linn

Fig 7: Anti-inflammatory assay of column eluted sample

***In vitro* Ant diabetic activity**

Alpha amylase inhibition assay

The intestinal digestive enzyme alpha-amylase plays avital role in the carbohydrate digestion. Antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha- amylase enzyme. The *In vitro* alpha amylase inhibitory studies demonstrated that *Mesua ferrea* Linn has well Antidiabetic activity. Column eluted fraction showed maximum inhibition of fraction No.3 84.93%at conc.500µg/ml and fraction No. 5 66.14% at conc.500 µg/ml. While reported that Antidiabetic activity of *Mesua ferrea* Linn seed is 67.52% at conc. 640 µg/ml. Dependant% inhibition listed in Table 4.6

Table 7: *In vitro* alpha amylase inhibition method

Concentration in µg/ml	Fraction 3		Fraction 5		Standard	
	Abs.	% inhibition	Abs.	% inhibition	Abs.	% inhibition
100	0.361	43.30±0.004	0.171	34.60±0.005	0.027	43±0.05
200	0.794	58.63±0.004	0.174	50±0.002	0.032	59±0.05
300	1.114	75±0.004	0.239	53.03±0.004	0.053	74.42±0.06
400	1.415	82.46±0.005	0.251	66.14±0.004	0.057	82.23±0.06
500	1.323	84.98±0.003	0.286	70.71±0.003	0.075	85.75±0.08

Values are expressed as mean±SD; Experimental group were compared with control

**p<0.01, considered extremely significant.

Fig 8: *In vitro* alpha amylase inhibition assay of column eluted fractions

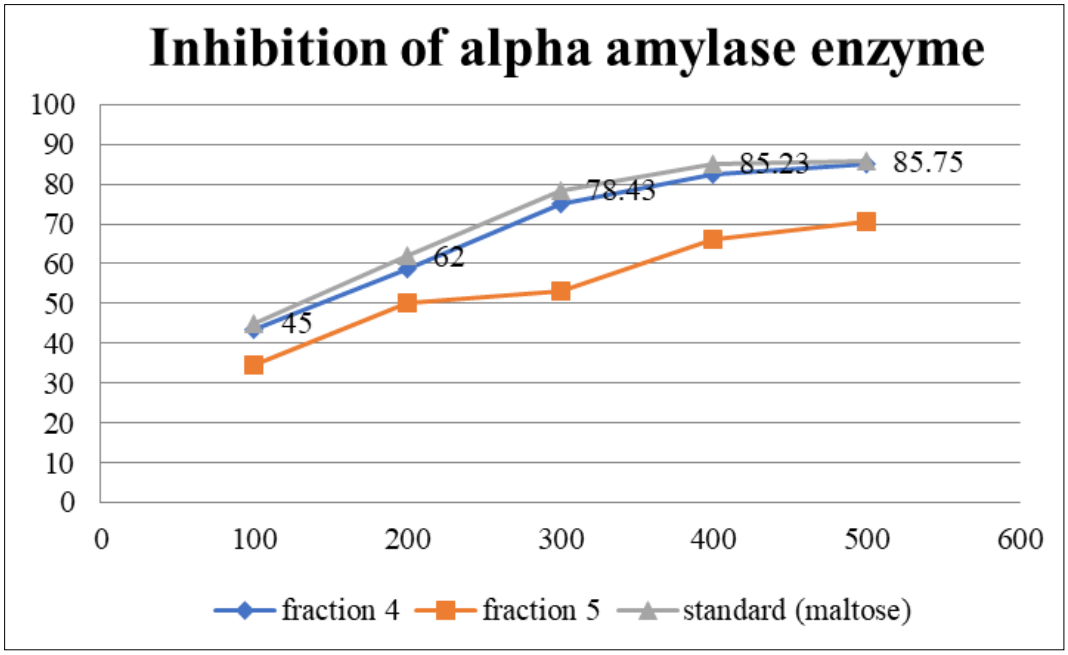

Fig 9: Graphical representation of *In vitro* alpha amylase inhibition assay of column eluted fractions of Nagkesar using maltose as a standard

Conclusion

The study's purpose was to describe the *in vitro* pharmacological activity of *Mesua ferrea* Linn. In a thin layer chromatographic study, n-butanol, acetic acid, and acetone were utilised as various solvent systems with varying polarity. TLC profiling reveals a pure band of peptide at 254 and 366 nm. *In vitro* anti-inflammatory effectiveness was evaluated using proteinase inhibitory activity, membrane stabilisation test, and albumin denaturation. Using aspirin as the reference drug, the anti-inflammatory activity fractions with the highest activity against albumin denaturation, membrane stabilisation assay, and proteinase inhibitory activity were examined. The Alpha Amylase Inhibition Assay was used to assess antidiabetic effectiveness *in vitro*. Fractions 3 and 5 displayed the most activity. Using reducing power, an antioxidant activity test was performed. Fraction no. 3 showed a higher absorbance of 0.94 at 500 g/ml.

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