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Phytochemical profile, *in-vitro* antioxidant, anti-diabetic and anti- inflammatory activities of traditionally used *Euphorbia hirta* (L.) growing under wild conditions of Northern Punjab

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Euphorbia spp., are the widely distributed and traditionally used herbal plants due to their diverse biological activities. The aim of this study was comparative extraction of secondary metabolites with different solvents like acetone, ethanol, methanol and water and assessment of their biological activities from *Euphorbia hirta* L. Plant extracts from dried powder were prepared in different solvents. Phytochemicals like: phenolics, flavonoids and condensed tannins were estimated using standard assays. GC-FID studies were conducted to find out the presence of various bioactive compounds. Fingerprint analysis using UV, FT-IR and fluorescent spectroscopy was also conducted. Antioxidant activities using various assays, anti-diabetic and anti-inflammatory potential of extracts were evaluated. Fingerprint analysis using, UV-, FT-IR and fluorescent spectroscopy specified the occurrence of adequate level of polyphenolics in all solvent extracts. Amongst all solvents acetone gave higher yield of polyphenolics *in-toto*. GC-FID analysis of acetone extracts depicted the presence of bioactive compounds like major 1-naphthalenepropanol. All extracts depicted enough level of antioxidant activities viz: DPPH, ABTS, hydroxyl radical, iron chelating and lipid peroxidation, but in a solvent dependent manner. Adequate anti-diabetic activity and anti-inflammatory activities were also observed in extracts. The results specified that by using suitable solvents bioactive compounds from *Euphorbia* species can be extracted and used as therapeutic agents having potential biological activities.

Key words: antioxidants, anti-inflammatory, anti-diabetic, *Euphorbia hirta*.

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Introduction

The use of medicinal plants has a long and rich history that dates back to the Vedic period, around 3500-1600 BC. This period is considered the dawn of Indian civilization. During this time, Ayurveda and Unani medicine, the two most ancient systems of traditional medicine in India, were developed (1). The use of medicinal plants was not limited to India, but it was also prevalent in many other ancient civilizations, including Egypt, Greece, and China (1). According to Ayurveda and Unani literature, medicinal plants were the foundation of all medical advancements during that time. These plants were used extensively in the treatment of various ailments and health conditions, and their effectiveness was well-recognized. Medicinal plants played a crucial role during the Vedic period and used as an essential aspect of a traditional and complementary medicine. Aromatic and medicinal plant parts, such as shoots, leaves, roots, and seeds, are the major sources of therapeutic agents in these practices (2). They were used for medicinal purposes, food, cosmetics, perfumes, and religious ceremonies. Alternative and complementary medical practices have been gaining popularity worldwide due to the limitations of conventional medicine and the growing demand for natural and holistic treatments. Aromatic and medicinal plants contained various active compounds, including essential extracts and products, flavonoids, alkaloids, and terpenoids, which are chemically complex and constitute a diverse mixture of molecules that possessed pharmacological properties and exhibited therapeutic effects on the human body (3).

Euphorbia hirta L. (also known as asthma-plant, Image 1) is a member of the *Euphorbiaceae* family, which is the third largest family of flowering plants with 1600 recognized species (4). This genus has distributed all over the world are found in hotter and tropical regions including America, China, India and Australia. This plant grows well along roadsides, grasslands, open fields and pathways. More than 5% of species of *Euphorbia* are used in traditional medicines, such as vomiting and cleaning agents. *Euphorbia* is widely used to treat asthma, digestive and respiratory disorders, skin and inflammatory conditions, hypertension, and bacterial cures (5). Herbal tea made from this plant is consumed as traditional medicine in the Philippines (where it is known as *tawa-tawa*), to cure dengue fever and malaria. This plant also exudates an abundant white latex which poses immense biological activities such as antifertility, sedative, anthelmintic,

anxiolytic, analgesic, antiasthmatic, and antispasmodic (5, 6). Extracts of *E. hirta* have been showed for anticancer activity (7). In the Philippines, *E. hirta* is one of the medicinal plants currently being used for its potential against coronavirus (COVID-19) (8-10) Biological potential of *Euphorbia* spp. are due to having diverse range of secondary metabolites such as phenolics and flavonoids, alkaloids, triterpenoids, and, amino acids, (11, 12). Thus, the aim of this study was solvent-dependent extraction of secondary metabolites and evaluation of total antioxidant activity, anti-diabetic and anti-inflammatory analysis from leaves of *E. hirta.*

Image 1. Pictorial view of *E. hirta*.

Experimental section

Collection of Plant Sample

The plant sample of *Euphorbia hirta* L. was collected growing under the natural conditions, from the CSIR aroma nursery areas of Lyallpur Khalsa College, Jalandhar, (71°-31° east latitude and 30°-33° north longitude). The plants were identified by botany department and voucher specimen bearing number BT 110 was deposited. The whole plant sample of *Euphorbia hirta* was collected, washed with distilled water and oven dried at 40°C**.** The dried plants were pulverized to powder by analytical mill and stored in aluminum foils at -20°C for further processing.

Preparation of plant extracts

For preparing plant extracts, four different solvents viz: water, methanol/water (8:2, v/v), acetone /water (8:2 v/v), and ethanol/water (9:1, v/v) were used. About 3g of dried powder was mixed with 30 mL of each solvent and put on magnetic stirring for 30 min at room temperature and left overnight in solvents at room temperature (37°±8°C). The crude extracts were subjected to centrifugation at a speed of 10,000 rpm for 10 minutes at 37°±8°C. After the centrifugation, the supernatant was collected and stored for 24 h at 4°C.

Preliminary test (Qualitative analysis of phytochemicals)

Preliminary detection of various phytochemicals dissolved in various extract was assayed following Sharma et al (13).

Phytochemical analyses

The presence of various phytochemicals like phenolics, flavonoids and tannins in extracts was done following the protocols of Sharma et al (13). All extracts produced have been analyzed in the proposed tests for comparison purposes.

Total phenolic content

500 µl of *Euphorbia* extract were mixed with 3ml of distilled water and 0.5ml of Folin-Ciocalteu reagent was added. The mixture was shaken well and incubated at room temperature (37°±8°C) for 5 minutes. After that, 2 ml of 20% (w/v) Na_2CO_3 was added, and the mixture was incubated in the dark for 1 hour at room temperature (37°±8°C). The absorbance of the mixture was measured at 650 nm, and distilled water was used as a blank. Gallic acid was taken as the standard, and the results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DWT). All samples were analyzed in triplicate.

Total flavonoid content analysis

To 0.5 ml of *Euphorbia* extract, 1 ml of methanol and 4 ml of distilled water were added and incubated for 5 min at room temperature (37°±8°C). After incubation, 0.3 ml of 5% (w/v) $NaNO_3$ and 0.3 ml of $AlCl_3$ were added and again incubated in the dark for 15 min at room temperature (37°±8°C). Absorbance was determined at 510 nm using Rutin as the standard curve, and results were expressed as milligrams of Rutin per gram of dry weight (mg RUF/g DWT). All samples were analyzed in triplicates.

Total condensed tannins analysis

To 0.5 ml of the *Euphorbia* extract, 3 ml of 4% vanillin solution and 1.5 ml of concentrated HCl were added and mixed well, followed by incubation for 15 min at room temperature (37°±8°C). After that, the absorbance was taken at 500 nm, with methanol being used as a blank. The total tannin content was expressed as milligrams of ascorbic acid per gram of dry weight (mg AA/g DW). All samples were analyzed in triplicates.

Spectral analysis of extracts

1 ml of *Euphorbia* extract was analyzed by UV-VIS spectrophotometric (Labtronics) in the range of 200-400 nm. UV spectra was recorded and analyzed. FT-IR study was conducted to identify functional groups in the active

constitutes of *Euphorbia* extracts. For this small amount (about 10µL) of *Euphorbia* extracts were utilized. The plant extracts were scanned in the range of 400-4000 cm^{-1}, and peaks were noted using FT-IR spectrophotometer (Perkin Elmer, USA). The fluorescence analysis of *Euphorbia* extracts was achieved using on fluorescent Spectrophotometer (FL6500, Perkin Elmer, USA). For this 3 ml of *Euphorbia* extracts were taken in quartz cuvettes and was excited at 380 nm.

In-vitro determination of Antioxidants

DPPH Scavenging Activity
To 0.5 ml of *Euphorbia* extracts, 2.8 ml of DPPH solution was added and incubated in the dark at room temperature (37°±8°C). Then the absorbance was observed at 517 nm, with 82% methanol being used as a blank and 3 ml of DPPH solution being used as a control. Ascorbic acid was taken as the positive control. All samples were analyzed in triplicates. The DPPH scavenging activity of *Euphorbia* extracts was determined in terms of using this formula, i.e. % scavenging = Abs. of control - Abs. of the sample x 100 / Abs. of control.

Hydroxyl Scavenging Activity
To 0.5 ml of *Euphorbia* extracts, 1 ml of 0.75 mM 1,10-phenanthroline, 2 ml 0.2 M sodium phosphate buffer (pH 7.4), and 1 ml of 0.075 mM $FeSO_4$ and 1 ml of H_2O_2 (0.01%) were added and incubated for 30 min at 37°C. Distilled water was used as a blank, and absorbance was noted at 500 nm. Ascorbic acid was used as the positive control. The hydroxyl scavenging activity was calculated by using this formula: Scavenging activity (%) = (Abs. of control - Abs. of the sample) x 100 / Abs. of control. All samples were performed in triplicates.

ABTS Scavenging activity
To 0.5 ml of *Euphorbia* extracts, 3ml of the generated ABTS cation solution was added. Incubate the reaction mixture at room temperature (37°±8°C) for 6min. The absorbance was measured at 734nm. Ethanol was taken as blank. Ascorbic acid was taken as positive control/standard. Pure ABTS radical solution was taken as control. The test was performed in triplicates. The percentage ABTS+ radical scavenging was calculated with the equation: ABTS radical scavenging activity (%) = $[(A_0—A_1)/A_0]$ x 100, where A_0 = Absorbance of the ABTS radical in the absence of sample/positive control; A_1= Absorbance of test sample/standard.

Iron Chelating Activity
To 0.5 ml of *Euphorbia* extracts , 900µl of 500µM $FeSO_4$ was added followed by addition of 78µl of 0.25% 1,10-phenanthroline. The absorbance of resulting solution recorded at 510nm against water as blank. Solution with $FeSO_4$ was taken as control. EDTA was taken as standard. The Fe^{2+} chelating ability of extract was calculated by using following formula. Chelating ability (%) = $[(A_{CONTROL} - A_{SAMPLE})/A_{CONTROL}]$ x 100.

Total Antioxidant Activity
To 0.5 ml of *Euphorbia* extracts, 1ml reagent solution (0.6 mM H_2SO_4, 28 mM Na_3PO_4, and 4 mM ammonium molybdate) was added in the test tube and incubated at 95°C for 95 min and cooled down at room temperature (37°±8°C). Absorbance was taken at 695nm. Ascorbic acid was taken as the standard curve, all sample was performed in the literature.

Lipid Peroxidation Activity
0.5 ml *Euphorbia* extracts was mixed with 0.25ml (10%) egg yolk, and 10 µl Fe_2So_4 followed incubation for 30 min at room temperature (37°±8°C). After adding 0.75ml glacial acetic acid and 0.75ml of thiobarbituric acid, the mixture was incubated for 1 hr at 37°C. After centrifugation at 3000 rpm, 1ml of supernatant was used for absorbance at 532 nm on a spectrophotometer. Inhibition of lipid peroxidation = [(Absorbance of control - Absorbance of sample) / Absorbance of control] x 100. All samples were performed in triplicates.

Alpha Amylase Inhibition (Anti-diabetic assay)
The *Euphorbia* extracts were taken in different test tubes in the range of 50 µl-250 µl. Then, 125 µl of the α-amylase solution (5 mg/ml) was added to each test tube along with 500 µl of sodium phosphate buffer (0.02M, pH 6.9). The mixtures were further incubated at 25°C for 10 minutes.
Next, 500 µl of 2% (w/v) starch solution was added to each test tube, and the mixtures were again incubated for 10 min at 25°C. To stop the reaction, 500 µl of DNS reagent was added to each test tube, and the mixtures were incubated at 100°C for 5 min in a water bath chamber. After the incubation period, the solution mixtures were diluted by adding 6 ml of distilled water to each test tube. The absorbance of each sample was then taken at 540 nm. The alpha-amylase inhibition activity was calculated using the formula: (%) inhibition = [(Absorbance of control - Absorbance of sample) / Absorbance of control] x 100. All samples were performed in triplicate.

Mode of Alpha-Amylase Inhibition
75 µl of *Euphorbia* extracts were taken with 250 µl of α-amylase solution in different test tubes and pre-incubated at 25°C for 10 minutes in one set. In another set, the α-amylase solution was placed in different test tubes and pre-incubated at 25°C for 10 minutes. Then, 250 µl of sodium phosphate buffer was added and incubated for 10 minutes at 25°C in a water bath chamber. After incubation, 2% starch solution (50 µl to 250 µl) was added in increasing order to both sets of test tubes. The mixture was then incubated for 10 minutes at 25°C and then boiled for 5 minutes. Finally, 500 µl of DNS reagent was added to stop the reaction. The

amount of reduced sugar was determined using the maltose standard curve. The mode of alpha-amylase inhibition of plant extracts was determined by Line-weaver Burk plot and Michaelis-Menten kinetics.

Anti-inflammatory Activity

Anti-inflammatory Activity using protein denaturation assay of extracts was determined following [8]. A mixture of 0.4 ml 1% BSA and 4.78 ml phosphate buffered saline was prepared. To this mixture, 100 µl of different *Euphorbia* extracts were added and incubated at 37°C for 15 minutes in a water bath. After incubation, the mixtures were cooled down and absorbance was measured at 600 nm against phosphate buffered saline as blank. In another set of experiments, the mixtures were incubated at 70°C for 5 min followed by cooling. Mixture was subjected to fluorescent spectroscopy analysis with excitation wavelength 280 nm and spectrum was noted in the wavelength range of 300-400 nm. A mixture of 0.4 ml 1% BSA and 4.78 ml PBS was used as a control. Combiflame (75 mg) was used as a standard. The percentage of inhibition of anti-inflammatory activity was calculated using the formula: (%) inhibition of Anti-inflammatory activity = [(Absorbance of control - Absorbance of sample) / Absorbance of control] x 100. All experiments were performed in triplicate.

Gas Chromatography

The Gas Chromatography (GC-FID) study of acetone extract of *E. hirta* was performed by using a Chemtron 2045 gas chromatograph coupled with flame ionization detector. A stainless steel column (2 m long) filled with 10% OV-17 on 80-100% mesh Chromosorb W (HP) was used. Nitrogen gas was used as carrier gas a with flow rate of 30ml/min. The detector and injector temperature were kept at 200°C and 250°C. 0.2µl of sample was injected. Ramping conditions for oven were: 110°C maintained initially then ramped to 200°C at rate of 2°C /min. Bioactive compounds were identified by comparing the relative retention time with known standards or with data published in the literature.

Statistical analysis

MS Excel software (version 2010) was used to determine P values by Tukey test. Values of $P< 0.05$ were significant.

Results and discussion

Fingerprint analysis

Preliminary an analysis indicated the presence of phytoconstituents like flavonoids, phenolics and tannins in the extracts (Table 1). As illustrated in Table 1, Qualitative analysis of phytochemicals of all solvents like ethanol, acetone, methanol and water depicted the presence of flavonoids, phenolics and tannins. Whereas other metabolites like anthraquinones, reducing sugars, saponins, steroids, and terpenoids were not detected. UV-Vis spectroscopy is a screening tool which is often used to detect secondary metabolites (13-14). The UV-profile of all *Euphorbia* extracts in ethanol, methanol, acetone and water is shown in Figure 1A. UV-VIS Spectroscopy analysis of methanol, ethanol, and water containing extracts revealed major absorption maxima at about 260 nm with absorption range from 1.7 to 2.4. In acetone extracts, one major peak at 360 nm was detected. In UV-spectrum, the incidence of peaks in the region from at 260-360 nm is a clear indication of the presence of secondary metabolites/phytochemicals in all *Euphorbia* extracts. These annotations are in corroboration with earlier findings of Raji et al. (14) in *Euphorbia hirta, Cassia alata, Thespesia populnea,* and *Wrightia tinctoria* extracts while characterizing secondary metabolites. Authors observed UV spectral peaks in the range from 250-390 nm, indicating the presence of alkaloids, flavonoids, tannins and saponins. Authors pointed out that since most of the phytochemicals are having a complex structure in which the carbon is in a specific condensation state gives definite and unique absorption spectra mostly in the visible or UV region.

Table 1. Preliminary Phytochemical Analysis

Extract	Acetone	Methanol	Ethanol	Water
Anthraquinones	-	-	-	-
Flavonoids	+	+	+	+
Reducing sugars	-	-	-	-
Saponins	-	-	-	-
Steroids	-	-	-	-
Tannins	+	+	+	+
Terpenoids	-	-	-	-
Phenols	+	+	+	+

Figure 1B depicts the fluorescent emission spectra of *Euphorbia* solvent extracts. Various bioactive components when excited with suitable light emit fluorescence (15). In all extracts, one major peaks lambda near 670 was detected, indicating that fluorescent substances are present in *Euphorbia* solvent extracts. Maximum extraction of phytochemicals was observed with ethanolic and acetone containing *Euphorbia* extracts. Other solvents like water unable to extract noticeable level of florescent compounds. UV-profile also validated the same notion. It was reported that fluorescent candidates at lambda near 670 nm may be due to accumulation of polyphenolics compounds like anthocyanins, phenolics, alkaloids and aromatic benzenoids

are (15). FT-IR spectrum of *Euphorbia* extracts is displayed in Figure 1C. Differences in FT-IR peaks are given in Table 2. All solvents extract displayed variation in FT-IR peaks. But, methanol and ethanol extracts FT-IR profile was relatively similar. More number of peaks was detected in acetone extracts and least in water.

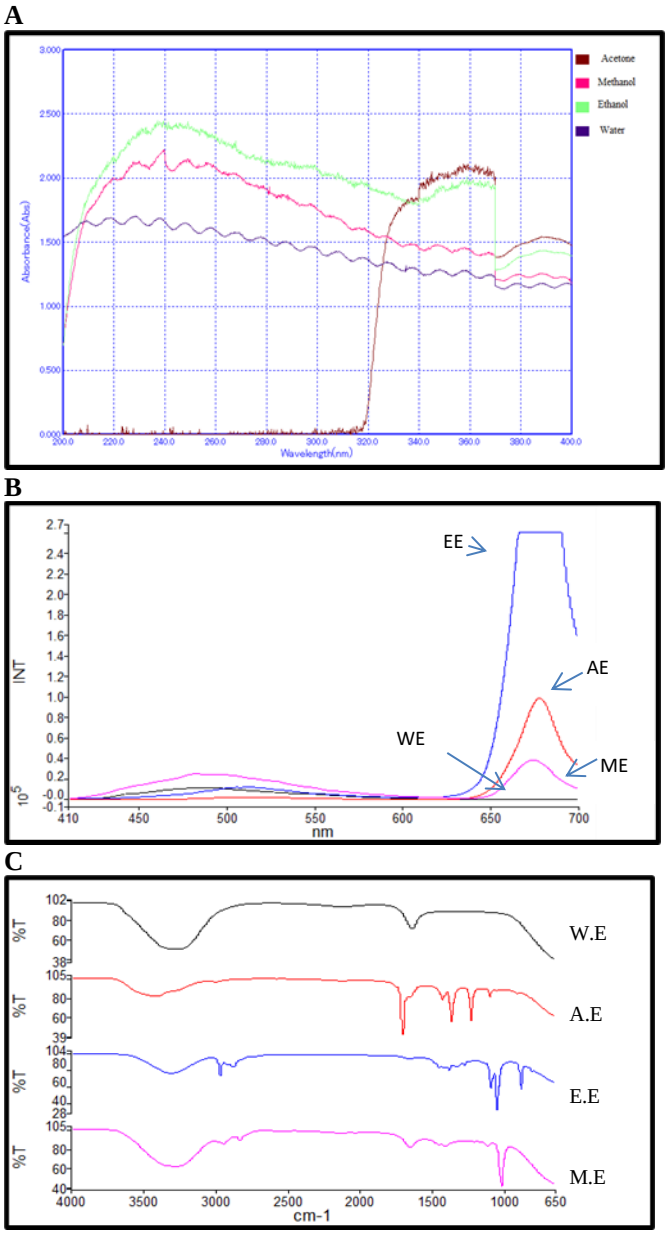


Figure 1: Fingerprint analysis of *E. hirta* extracts. A: UV, B: Fluorescent and C: FT-IR, abbreviations used: AE: acetone extract, EE: ethanol extract, ME: methanol extract, WE: water extract.

A broad band in the range of 3400 to 3200 cm^{-1} was detected in FT-IR spectrum of all extracts which was due to OH- group, hence indicating that occurrence of phenolic compounds. In ethanolic and methanolic extracts, another sharp band at about 2800-2900 cm^{-1} was detected due to C-H stretching due to alkanes. Peaks in the range of 1700 cm^{-1} were observed in acetone extracts, indicated the presence of anhydrides, esters, aldehydes, carboxylic acids, ketones and amide. Variations were observed in the 1500-400 cm^{-1} region, which is also known as fingerprint region of FT-IR spectra. It is the 4th region in the IR spectrum, which is known as major region as it comprises a huge number of complex peaks (16). For example: in acetone extracts peculiar major bands in the range of 1361-1226 cm^{-1} were detected, that were due to C=C stretching of alkenes, which were absent in other extracts. In the range of 1014-1081 cm^{-1}, minor bands were detected ethanolic and methanolic extracts due to C-N stretch of aliphatic amines. One minor peculiar band in the range of 879 cm^{-1} was detected only in ethanolic extracts which was due to 1,2,3-tridistributed. All these compounds belong to the secondary plant metabolites (14). These results infer that all *Euphorbia hirta* is rich source in secondary metabolites. The occurrence of afore-mentioned secondary metabolites might be the reason for medicinal properties of *Euphorbia hirta*.

Antioxidant analysis of Euphorbia extracts

Due to richness of variety of bioactive components *Euphorbia* spp. are enormously used as therapeutic agents in traditional medicine (17). Our findings, as shown in Table 3, showed that the phenolic concentration in the acetone extract was 0.36 mg GAE/g DWT, while it was only 0.06 mg GAE/g DWT in the methanolic extract. The phenolic content of the water extract was 0.33 mg GAE/g DWT, whereas the phenolic content of the ethanolic extract was 2.5 mg GAE/g DWT. In the investigation, it was found that the ethanolic extract of *Euphorbia hirta* contained more total phenols than the extracts made from methanol, acetone and water. The acetonic extract's flavonoid concentration was determined to be 3.8 mg RUE/g DWT, compared to 1.5 mg RUE/g DWT in the methanolic extract. The ethanolic extract's flavonoid concentration was found to be 0.27 mg RUE/g DWT. With 8.2 mg RUE/g DWT, the water extract had the highest flavonoid content. The tannins amount was determined to be 26.0 mg AA/g DWT in the acetonic extract, 11.0 mg AAE/g DWT in the methanolic extract, 1.0 mg AAE/g DWT in the ethanolic extract, and 11.0 mg AA/g DWT in the water extract. It was determined that in *Euphorbia hirta*, acetone extract had a higher tannin concentration than methanol, ethanol, and water extracts. Earlier studies revealed the presence of flavonoids, triterpenoids, alkanes, amino acids, and alkaloids in other species of *Euphorbia* like *E. ingens*, *E. mey*, *E. tirucalli*, and *E. triangularis* (5). Over all, *in-toto*, the net amount of phytochemicals was more in acetone extracts than other solvents. This finding was in agreement with previous report of Medini et al (17) in *Limonium delicatulum* advocating that acetone was best solvent to extract bio-molecules. Similar solvent dependent differences in the

amounts of phytochemicals have been reported in medicinal plants (18). Medeni et al., (17) described that abstraction of polyphenols is extremely reliant on on type of solvent used, its polarity. In addition, several other factors like: phenolics polymerization, and their interactions, biological factors such as organ, genotype, and edaphic and environmental factors cannot be ruled out. Henceforth the variances in polarity of solvents used in this study might be the cause for alterations in extraction yield and antioxidant activity.

Table 2: FT-IR peaks detected in various extracts of *Euphorbia hirta*.

Functional group	**Compound**	**Frequency range [cm^{-1}]**	***Euphorbia* hirta [cm^{-1}]**			
			Acetonic extract	Methanolic extract	Ethanolic extract	Water extract
O-H stretch	alcohol	3550-3200	3437.92	-----	3314.38	3294.22
C-H stretch	alkane	3000-2840	-----	2949.91	2973.44	-----
C=C		2840-2720	-----	2837.76	-----	-----
C=O stretch	aldehydes, ketones, esters, carboxylic acids, anhydrides	1830-1650	1702	-----	-----	-----
C=C stretch	alkene	1662-1626	-----	1650.09	-----	1637.57
C=C stretch	α,β ketone	1620-1610	-----	------	-----	-----
O-H bending	alcohols	1420-1330	1361.77	1409.58	1379.58	-----
C-H bending	alkanes	1450-1465	------	-----	-----	-----
O-H Bending	phenols	1390-1310	1361.77	------	1379	-----
C-N, C=O stretch	aliphatic amines, aromatic	1280-1020	1226.95	1113.25	1274.40	-----
S=O stretch	sulfoxide	1070-1030	-----	-----	1045.34	-----
C-O stretch	alcohol	1100-1010	1092.88	1014.34	1087.44	-----
C-H bending	1,2,3-tridistributed	780±20	-----	-----	879.72	-----

Table 3**:** Total phytochemical content of the different extract.

Extract	**Acetone**	**Methanol**	**Ethanol**	**Water**
TPC [µg GAE/g DWT]	0.36±0.02a	0.06±0.01a	2.5±0.02a	0.33±0.04a
TFC [µg RUE/g DWT]	3.82±0.03b	1.50±0.02b	0.27±0.03b	8.24±0.03b
TTC [µg AAE/g DWT]	26.0±0.58c	11.0±0.09c	1.0±0.02c	11.0±0.08c

Different letters within row indicates significant difference at P≤0.05 with in column, (mean ± SD, n=3), TPC: total phenolic content, TFC: total Flavonoid content, TTC: total tannin content, DWT: dry weight, GAE: gallic acid equivalents, RUE: Rutin equivalents, AAE: ascorbic acid equivalents.

To unravel therapeutic potential of any medicinal plant formulation, the antioxidant potential is the key component generally employed to studied (19). Since, a plant possess a complex mixture of bioactives and henceforth it is very problematic to measure the antioxidant potential of individual component. Consequently, six different methods viz: DPPH scavenging activity, ABTS scavenging activity, Hydroxyl scavenging activity , Total antioxidant activity , Iron chelating activity, Lipid peroxidation activity measurements were implemented to assess antioxidant potential of *E. hirta* solvent extracts (Figure 2).

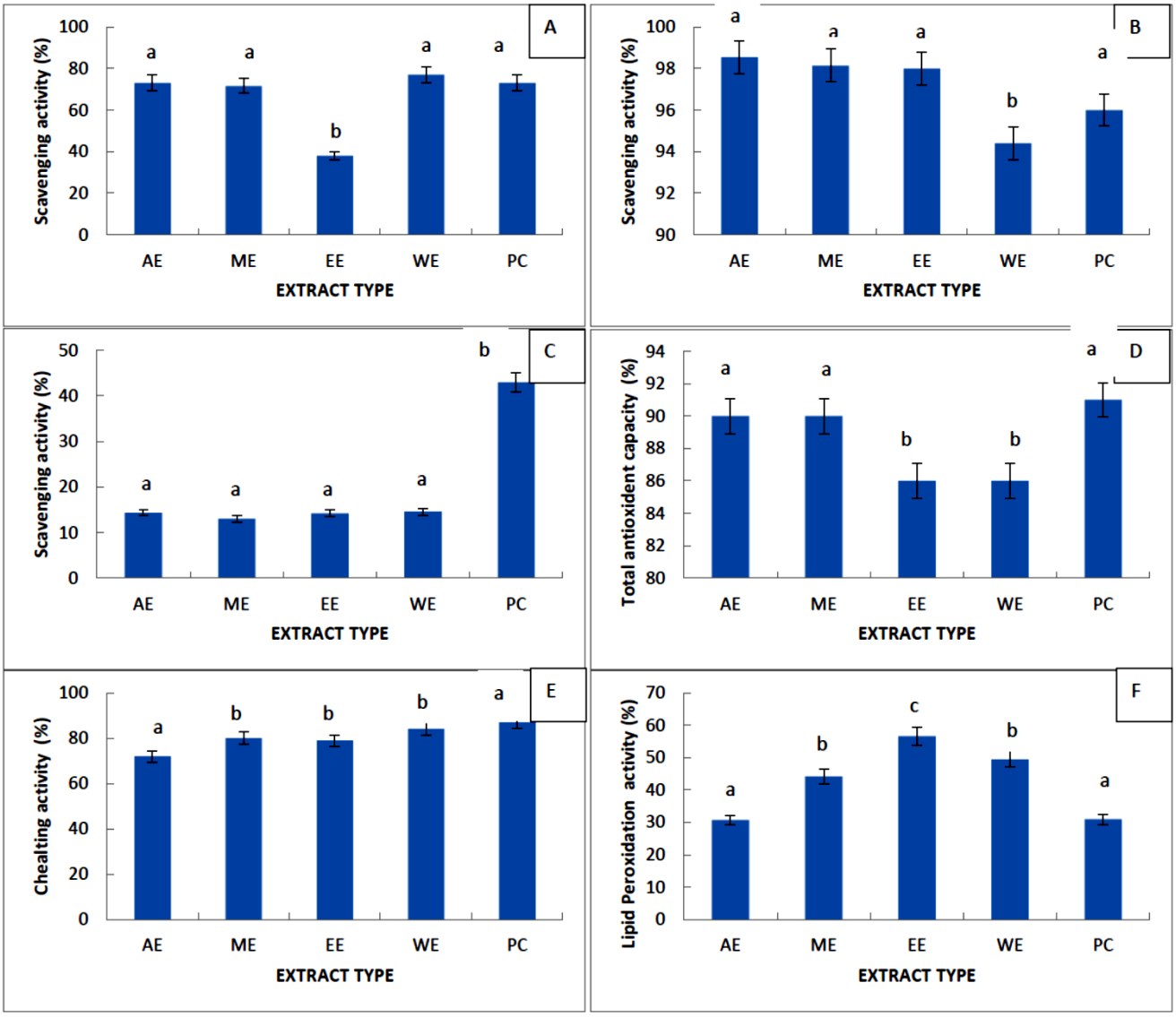


Figure 2: Various antioxidant activities of *Euphorbia hirta* solvent extracts. DPPH scavenging activity (A), ABTS scavenging activity (B), Hydroxyl scavenging activity (C), Total antioxidant activity (D), Iron chelating activity (E), Lipid peroxidation activity (F). Abbreviations: AE: acetone extract, ME: methanol extract, EE: ethanol, extract, WE: water extract, PC: positive control (ascorbic acid in panel A, B, C, D, F). PC: positive control (EDTA in panel E). [a,b] different letters shows significant difference among each other at P≤0.05 with in panel.

In the present study, different solvent extracts of an *E. hirta* were used to quantify the DPPH scavenging activity. The stable free radical DPPH can discolour when there are antioxidants present. It is the most common, straightforward, and trustworthy approach for determining antioxidant activity. The odd electron that makes up the DPPH radical is responsible for its striking deep purple colour and absorption (19). It decolorizes by acquiring an electron from an antioxidant, which may be detected by a drop in absorbance. Maximum scavenging activity was observed with water extract exhibiting 76% activity. However, ethanolic extract exhibited a lower DPPH activity of 38%. All other extracts depicted scavenging activity in the range from 71-73% which was not significantly different to acetone extract. Khan et al (19) observed strong DPPH scavenging and antioxidant activities in methanolic extracts in *Launaea procumbens.* Earlier study by Al-Snafi and Esmail (20) on *Euphorbia hirta* also demonstrated

DPPH scavenging activity in the range of 44-74% in methanolic extracts of leaves, flowers, roots. In the present study, the ABTS scavenging activity of different solvent extracts in the presence of plant extract was quantified by measuring the remaining radical cation concentration after reaction with antioxidant compounds. ABTS, a blue chromophore product, was produced by the reaction between ABTS and potassium persulfate (18,19). In the presence of the plant extract or trolox, ABTS reacts with potassium persulfate to create ABTS+•, a blue chromophore. Reduced is the preformed cation radical and the leftover radical cation concentration was then measured following the reaction with the antioxidant agent (18). All extracts except water depicted appreciable level of activity in the range of 98%. The water extract activity was 94%. The results showed that the acetone extract of *E. hirta* exhibited remarkable ABTS scavenging activity. Hydroxyl radical (OH^-) is one of the vastly reactive and damaging species in free radical, able to damage all molecules formed in a cellular system (20). It induces severe damage to biomolecules, but also causes breakage of DNA strands and Denaturation of DNA which leads to carcinogenesis, cytokinesis and mutagenesis (19). The results showed that all extracts depicted almost equal hydroxyl scavenging activity in the range of 14%. Al-Snafi and Esmail (20) on *Euphorbia hirta* also demonstrated hydroxyl radical scavenging activity in the range of 83% in methanolic extracts of leaves. Total antioxidant activity (TAA) was a measure of the ability of a substance to scavenge free radicals and prevent oxidative damage to cells and tissues (20). Plants were a rich source of antioxidants and could scavenge free radicals and prevent oxidative damage. Maximum total antioxidant activity was observed with acetonic and methanolic extract which was in the range of 90%. While ethanolic and water extract showed 86% scavenging activity. In the experiment, the iron chelating activity of plant extracts was measured using EDTA as a standard. The results showed that the iron chelating activity of the methanolic, ethanolic and water extracts was significantly equal in the range of 79-84%, whereas acetone extract exhibited 72% activity. Chelating compounds create -bonds with metals and work as secondary antioxidants because they lower the redox potential, which stabilizes the metal ion's oxidized state (18, 19). Lipid peroxidation was a process in which free radicals attacked polyunsaturated fatty acids in cell membranes, resulting in the production of lipid peroxides (20). Lipid peroxidation caused damage to cell membranes, leading to various diseases and aging. The potential of plant extracts as inhibitors of lipid peroxidation was of great interest to the food and pharmaceutical industries. Additionally, the inhibition of lipid peroxidation was important for the prevention of various diseases, such as cardiovascular disease. Maximum total lipid peroxidation activity was observed with ethanolic extract with 56% value followed acetone and water extract exhibiting values in the range 44-49%. Least activity was observed with acetone extract exhibiting 34%. EDTA was used as a standard, and its activity at a concentration of 10µl was found to be 31%. Earlier research on *Euphorbia hirta* by Sharma et al (19) also reported appreciable antioxidant activities using *in vitro* assays using DPPH, ABTS, hydroxyl radical scavenging activity and Lipid peroxidation inhibition potential depicting free radical scavenging activity of 247 µmol Trolox equivalent per gram crude extract and IC 50 value 0.175 mg crude extract / ml, IC 50 value 0.162 mg crude extract/ml, and IC50 value 0.143 mg crude extract per ml, respectively. Mostly different herbal formulations and therapeutics drugs are used to mitigate oxidative stress and free radicals due to high scavenging power (19). We consequently opine that the noticeable antioxidant activities of *E. hirta* extracts may be attributed to the occurrence of phenolics, flavonoids and other compounds as detected in this study. Biological herbal extracts having high antioxidant activities is the symbol of its latent use in biological system as food supplement to mitigate damage of biomolecules by constraining free radicals and therefore rejuvenates the body functions (1). Previously the documentation of polyphenolic based bioactives from diverse plants extracts has become a significant area of health- and medical-related research (21).

Anti-diabetic potential of Euphorbia extracts

Plant extracts having alpha-amylase inhibition potential was of inordinate interest to food and pharmaceutical industries. Alpha-amylase inhibitors are compounds that could reduce the rate of carbohydrate digestion, which was useful for controlling blood sugar levels in people with diabetes (22). This is an attempt to search for alternative medicinal plants based drugs with increased potency and lesser adverse effects than synthetic drugs (23). In this study effect of *E. hirta* plants extract on alpha-amylase activity was studied. All extracts of *E. hirta* showed dramatic inhibition in α-amylase activity in solvent-dependent manner (Figure 3A). Maximum anti-diabetic potential was observed with methanolic extract (91% α-amylase inhibition activity) by using 500 µl of plant extracts. While other solvents α-amylase iinhibition activity ranged from 58-62%. These findings are in corroboration with earlier reports highlighting the α-amylase inhibitory activities from medicinal plant extracts (24). Using in-vivo models, in Wistar rats Kumar et al, (5) observed potent anti-diabetic activities in ethanolic and petroleum ether extracts in *E. hirta* flower extracts. Substantial anti-diabetic activities of hydro-alcoholic extract of *E. neriifolia* has also been cited using in-vivo models by Devi et al., (25). This study results suggest that *E. hirta* extracts exhibited its hypoglycemic potential which may be attributed to the occurrence of flavonoids and phenolics. Flavonoids were known to inhibit alpha-amylase activity by binding to the active site of the enzyme, while tannins inhibited alpha-amylase by forming complexes with the enzyme (24). The mode of alpha-

amylase inhibition activity of plant extract was determined by using the Michalis-Menten plot and Line-weaver Burk plot (Supplementary Figure 1), which showed that all extracts displayed the competitive inhibition. It indicated that extracts contained the most potent alpha-amylase inhibitors. The competitive inhibition from Michalis-Menten and Line-weaver Burk plot point to the fact that the active components in the extract do compete with the substrate for binding to the active site to retard the conversation of disaccharides to monosaccharides (23).

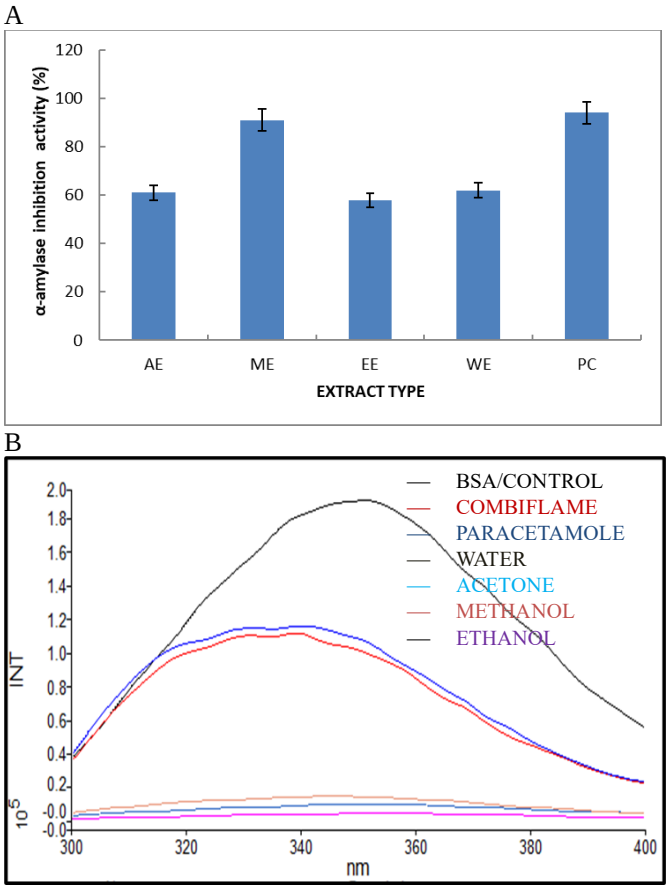


Figure 3: Alpha-amylase inhibition activities (A) and anti-inflammatory (Protein denaturation) activity (B) of various E. hirta based extracts.

Anti-inflammatory potential of *E. hirta extracts*

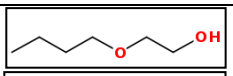
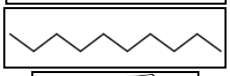
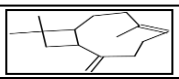
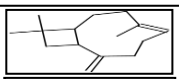
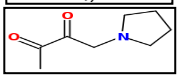
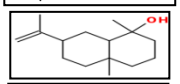
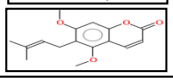
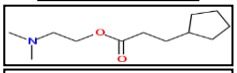
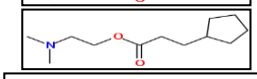
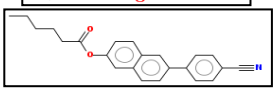
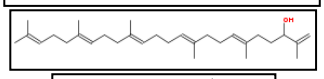
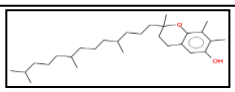
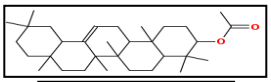
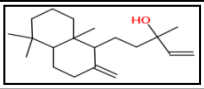
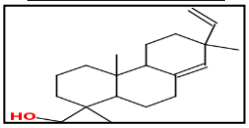
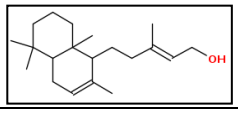
Protein denaturation is thriving recognized cause of tissue inflammation that is related with indications like swelling, pain, redness, and heat (26). Protein denaturation is the process in which native proteins lose their secondary and tertiary structures. The main motive behind loss of protein functions is the disturbance of hydrophobic, hydrogen and disulphide bonds in protein structures (26). Henceforth it was inferred that bioactive compounds that are capable to avert these changes and inhibit heat induced protein denaturation have prospective to be used as therapeutic anti-inflammatory drugs (27). In this study protein denaturation inhibitory activity of *E. hirta* plant extracts was examined by tryptophan fluorescent spectroscopy technique (13). Typical fluorescence spectra for denatured BSA and *E. hirta* extract under diverse solvents are shown in Figure 3B. as observed in Figure 3B, *E. hirta* acetone, methanol and ethanol and water extract formulations protected BSA against protein denaturation, as fluorescence intensity decreased considerably from 1.8x105 INT units to 0.2 x105 INT units after the addition of *E. hirta* extracts to BSA. Among all solvents, the maximum anti-inflammatory potential was observed for water extracts as about 9-fold decrease in fluorescence intensity was observed compared to other solvents. When compared with synthetic drugs paracetamole and combiflame, these drugs exhibited only marginal anti-inflammatory potential as fluorescence intensity decreased from 1.8 x105 INT units to 1.2 x105 INT units after the addition of drugs. These outcomes evidently specify the potential of *E. hirta* extract as anti-inflammatory agents. The anti-denaturation potential may be attributed to complex mixtures of polyphenolics having numerous compounds which may interact synergistically. These results were in consonance with earlier reports of Tatti et al. (28) who observed significant *in-vitro* anti-inflammatory activity in ethanolic extracts of *Butea monosperma*. *In-vitro* anti-inflammatory activity of ethanloic extracts of *Morinda umbellata* have been observed by Dharsana and Mathew (27).

Gas Chromatography (GC-FID) analysis

Phytochemical profile of *E. hirta* acetone extracts was also studied and shown in Figure 4 and Table 4. The bioactive compound identified by GC-FID analyses were enumerated along with their retention time and concentration. GC-FID analysis of acetonic plant extract showed the presence of 19 compounds, all identified 19 compounds were: 2-butoxy ethanol, decane, caryophyllene, 13-heptadecyn1-ol, 2-hydroxy-1-(1'-pyrrolidinyl)-1-buten-3-one, neointermedeol, 2h-1-benzopyran-2-one,5,7-dimethoxy, 3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester, hexanoic acid, 6-(4-cyanop henyl)-2-naphthalene ester, 17-pentatriacontene, 1,6,10,14,18,22-tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl, gamma-tocopherol, methyl commate b, olean-12-en-3-ol, acetate,(3.beta)-, 1-naphthalenepropanol, sandaracopimarinol, labd-7.13-dien-15-ol.acetate. The major component was 1-naphthalenepropanol with many small peaks indicating the presence of the bioactive compound in small quantities. *Euphorbia* spp have been reported to possess various potent biological activties like: anti-allergic activity, antibacterial activity, anti-diabetic activity, antitumor activity, anxiolytic and sedative activity due to richness of these kind of bioactive compounds (29). Due to these bioactive compounds, *E. hirta* plant extract has become a major part

of the research work, where they were used in the pharma sectors to discover new drugs (11).

Table 4: GC-FID analysis of bioactive compounds of acetone extract in *E. hirta.*

Peak no.	Retention Time (min)	Bioactive compound	concentration (%)	Structures
1	7.498	2- butoxy ethanol	0.7393	
2	8.248	decane	0.7541	
3	10.832	Caryophyllene	0.3108	
4	13.332	13-Heptadecyn1-ol	0.0872	
5	16.332	Caryophyllene	0.0202	
6	17.748	2-Hydroxy-1-(1'-Pyrrolidiyl)-1- Buten-3-One	0.0971	
7	20.332	Neointermedeol	0.5599	
8	24.248	2H-1-Benzopyran-2-One, 5,7-Dimethoxy	0.0217	
9	27.415	3-Cyclopentylpropionic Acid, 2-Dimethylaminoethyl Ester	3.8546	
10	29.582	3-Cyclopentylpropionic Acid, 2-Dimethylaminoethyl Ester	1.3061	
11	31.665	Hexanoic Acid, 6-(4-Cyanophenyl)-2-Naphthalenyl Ester	0.0139	
12	34.582	17-Pentatriacontene	0.3474	
13	35.415	1,6,10,14,18,22-tetracosahexaen-3-ol,2,6,10, 15,19,23-hexamethyl	0.4829	
14	36.748	gamma tocopherol	0.7315	
15	43.998	methyl commate B	2.7735	
16	46.332	olean-12-en-3-ol, acetate,(3.beta)-	4.8238	
17	50.665	1-naphthalenepropanol	21.4497	
18	55.665	Sandaracopimarinol	4.4059	
19	59.165	Labd-7.13-dien-15-ol. acetate	6.3063	

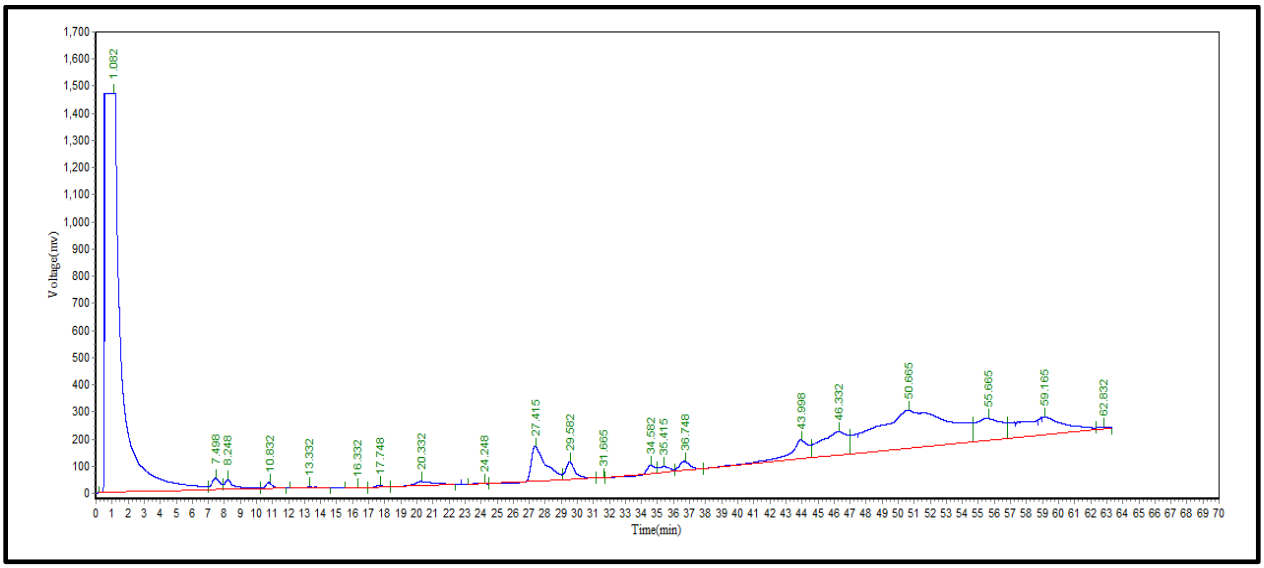


Figure 4: GC-FID analysis of acetone extract from *E. hirta.*

Conclusion

The present study evaluated phytochemical and biological potential of various solvent extracts in *Euphorbia hirta*. All extracts of *E. hirta*, especially acetone contained high levels of secondary metabolites like phenolics, flavonoids and tannins coupled with high antioxidant activity. GC-FID analysis also detected the presence of various bioactive molecules in the acetone extract. A strong anti-diabetic and anti-inflammatory activity was also noticed in methanolic, ethanolic and acetone extracts. It was observed that by choosing a selective solvent sufficient level of bioactive compounds having high biological activities could be extracted. This information can be used as a reference source of antioxidants in food and pharmaceutical based industries.

Acknowledgement

FIST lab for possible help in FT-IR and Florescent spectroscopy.

Conflict of interest

None to declare.

Supplementary section

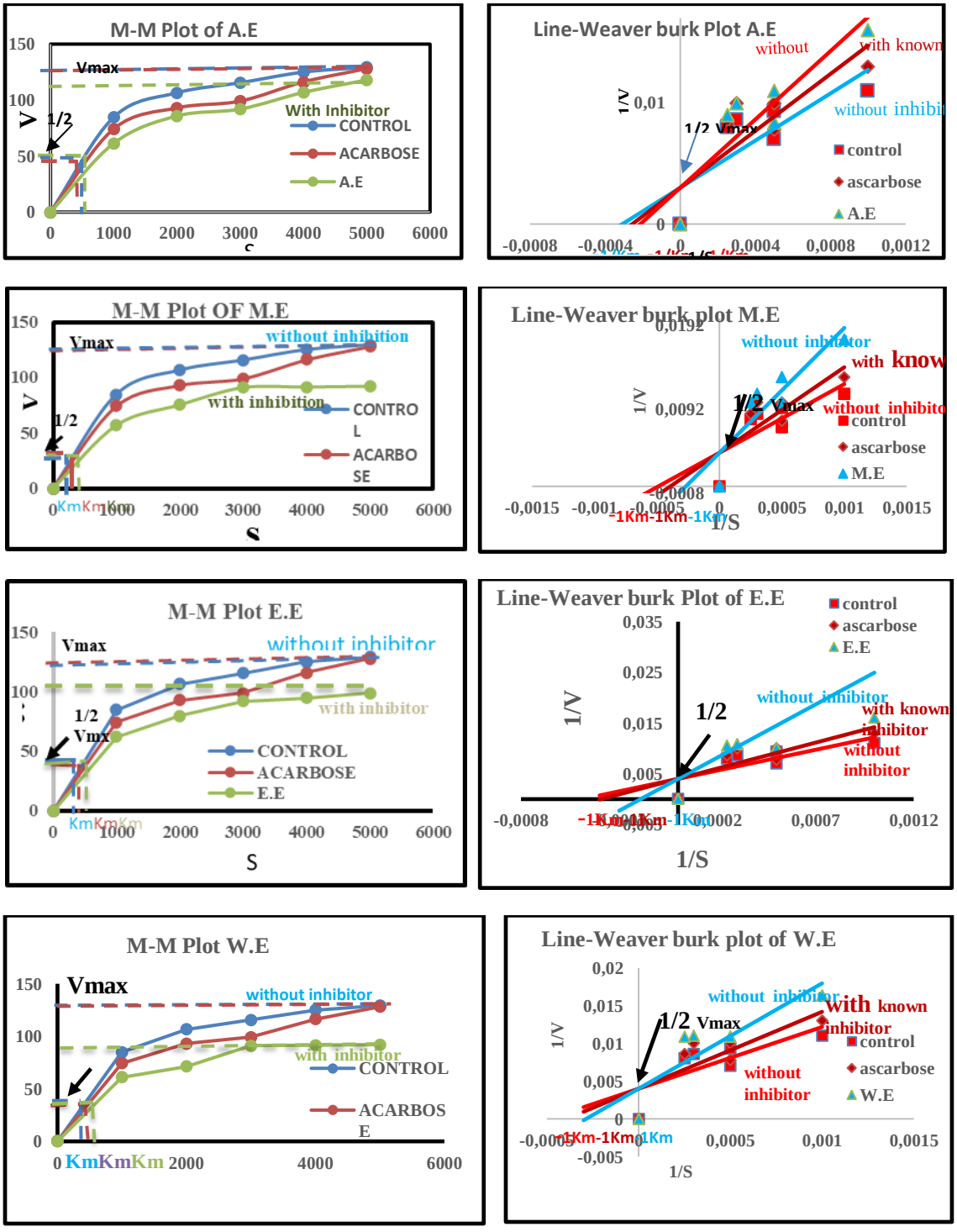


Figure1: Mode of α-amylase inhibition by Euphorbia extracts. A: acetone, B: methanol, C: ethanol and E: water.

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