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The Liver-Related Disease Curing Capabilities of Various Indigenous Plants Utilized by Ethnic Communities of Terai Belt of Dudhwa National Park, India

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Herbs carry on with decisive in the management of several liver disorders, despite the tremendous advancements in modern medicine. The current study was designed to identify liver related ailment curing potential of some unexploited plants, Cleome viscosa, Cordia dichotoma, and Tephrosia purpuria. Powders of all the selected plant parts were separately extracted using low, medium & high polar solvents using Soxhlet apparatus. Depending on the test used, numerous extracts could result in inconsistent outcomes due to their chemical complexity. Thus, a multi-assay method would be more instructive when assessing the antioxidant capacity of extracts. By measuring the total phenolic content and employing additional scavenging assay techniques (like DPPH radical, nitric oxide scavenging, hydroxy radicals scavenging, reducing power assay and Fe2+ chelating activity), the antioxidant properties of the crude extracts were ascertained. We have followed three time sample study techniques during these series of studies and the outcomes were contrasted with conventional antioxidants.
Keywords: polyphenols, Cleome viscosa, Cordia dichotoma, Tephrosia purpuria, reducing power.

1. Introduction

A variety of risk determinants, such as drugs, alcohol, radiation, and environmental contaminants, can cause oxidative stress in the liver, leading to severe liver issues like non alcoholic steatohepatitis and alcohol-induced liver damage. Using anti-oxidant options is a

sensible preventative and therapeutic approach for oxidative stress-related liver disorders (Li et al., 2015). It is increasingly evident that imbalances in the body's pro-oxidant and antioxidant homeostatic mechanisms are the root cause of the majority of ailments that afflict individuals today. The body's poor capacity to scavenge or quench free radicals due to dietary antioxidant depletion, elevated free radical generation, or severe oxidative stress from modern life is the main cause of pro-oxidant conditions. (Pizzino et al., 2017) Numerous diseases have been connected to damage by free radicals, such as diabetes, atherosclerosis, cancer, and liver cirrhosis. Lipid peroxidation and covalent binding are two ways that ROS-Reactive Oxygen Species demonstrated to damage tissues of human body. It has been shown that the lipid peroxidative pathway promotes collagen production as well as fibrotic scarring (Muriel, 2009). Oxidation is one of the most important processes in food, medicine, and even life systems that generate free radicals. When present in trace concentrations, antioxidants are substances that significantly slow down or stop the substrate from oxidizing. (Pham-Huy et al., 2008).

Through their interactions with free radicals and other reactive oxygen species within the body, antioxidants shield the organism from damaging oxidation events. Antioxidant therapy can therefore aid in preventing disorders linked to free radicals. Modern synthesized anti-oxidants (like BHA, BHT, TBHQ, propyl/octyl gallates etc.) should be replaced by naturally occurring antioxidants because they may have adverse health effects. (Lobo et al., 2010). For a long time, people have used plants and items made from them as medicine. Recent developments in medical research around the world have concentrated on the curative properties of plants since they have their strong antioxidant action, lack of side effects, and economic affordability. (Chaachouay & Zidane, 2024). Plants containing polyphenols demonstrated to have several biological benefits, including being anti-inflammatory, anticarcinogenic, antioxidants, and free-radical scavenging potentials (Panche et al., 2016). Novel natural antioxidants derived from specific plants have been the focus of in-depth research in this era as perfect antioxidants as well as radical scavengers.

Traditional medicine is still widely used, and plants continue to be a rich source of naturally occurring antioxidants that could lead to the creation of new medications. Antioxidants can be found using straightforward in vitro test methods. They can scavenge different types of free radicals and prevent several diseases. (Lourenco et al., 2019).

There are numerous models for evaluating antioxidant activity. The chemical complexity of the several extracts and compound mixes present may result in conflicting findings depending on the test employed. Thus, when evaluating the antioxidant capacity of extracts, a multi-assay approach would be more enlightening (Jung et al., 2024). This study evaluated each extract's total phenolic content in addition to its different free radical scavenging properties. The results were compared to those of traditional antioxidants.

Chemicals:

The analytical-grade chemicals and reagents, which included ammonium molybdate (NH4-2MoO), sodium phosphate (Na3PO4), sodium carbonate (Na2CO3), hydrogen peroxide (H2O2), potassium iodide (KI), sodium thiosulphate (Na2S2O3), ferrous sulphate (FeSO4), disodium hydrogen phosphate (Na2HPO4), potassium dihydrogen phosphate (KH2PO4), nitrogen-blue-tetrazolium (NBT), nicotinamide adenine dinucleotide & hydrogen (NADH) and dimethyl sulfoxide (DMSO), were all purchased from Sisco Research Lab in Mumbai,

India.

Estimation of total phenolic content:

The quantities of phenolic content were measured using Folin-Ciocalteu's phenol reagent (FCR) in Pet. Ether, ethyl acetate, and methanolic extracts of several plants (i.e., C. viscosa, C. dichotoma, and T. purpurea) in accordance with the accepted procedure (Molole et al., 2022). In a 100 ml Erlenmeyer flask, 46 milliliters of distilled water were mixed with 1 milliliter of the extract solution (containing 1 milligram) in methanol. 1 mL of the selected reagent was mixed to it and kept for three minutes. 2% of Na2CO3 (3 mL) was added to the mixture, and it was shaken sporadically for 2 hours at room temperature. At 760 nm, the absorbance was measured. The percentage of polyphenolic contents in each extract was determined by using below mentioned formula.

% of polyphenols = 0.001 × Pyrocatechol in microgram + 0.0033

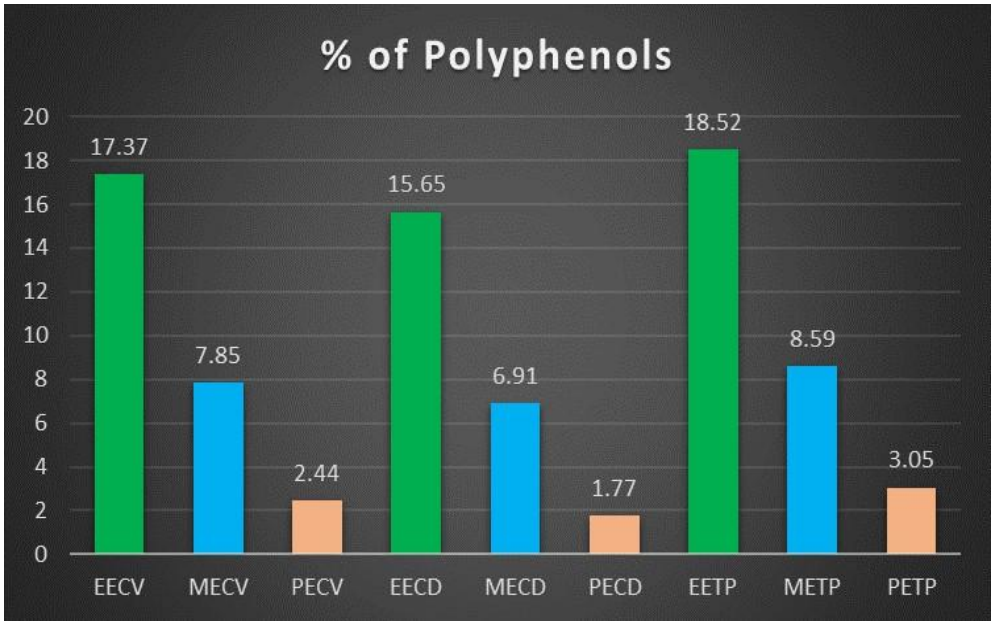

Figure 1 Total Polyphenolic Content in Various Extracts

During this study, we found that the polyphenol contents were quite high in all mid-polar solvent (i.e. ethyl acetate) extracts (EETP- 18.52 %, EECV-17.37 % and EECD-15.65 %). All the high polar solvent (methanol) extracts were having moderate polyphenolic contents (METP-8.59 %, MECV-7.85 % and MECD-6.91 %). The optimum content of polyphenols in all ethyl acetate extracts of our selected plants are reasons for their good liver curing possibility. The hydroxyl groups present in polyphenolics and flavonoids support all extracts their ability to reduce FC reagent (Molole et al., 2022).

Scavenging study by following DPPH method:

This is used for plant derived products for probable antioxidant potentials. The odd no. of electron of Nitrogen atom present in DPPH is brought down by obtaining a hydrogen atom from test drug i.e. plant extract to the corresponding hydrazine which can be observed through

spectrophotometer at 517 nm absorbance. In this study we have taken 0.1 mM DPPH soln in methyl alcohol. 1 mL of this soln was mixed with extract soln (3 mL) in water at various concn. These samples were incubated for 30 minutes in room temp. and absorbance was estimated in comparison to blank soln. Inhibition formula given below to find result.

$$\text{Inhibition Percentage} = 100\frac{\text{ABC} - \text{ABS}}{\text{ABC}}$$

ABC- Absorbance of control; ABS- Absorbance of sample

Results are represented below

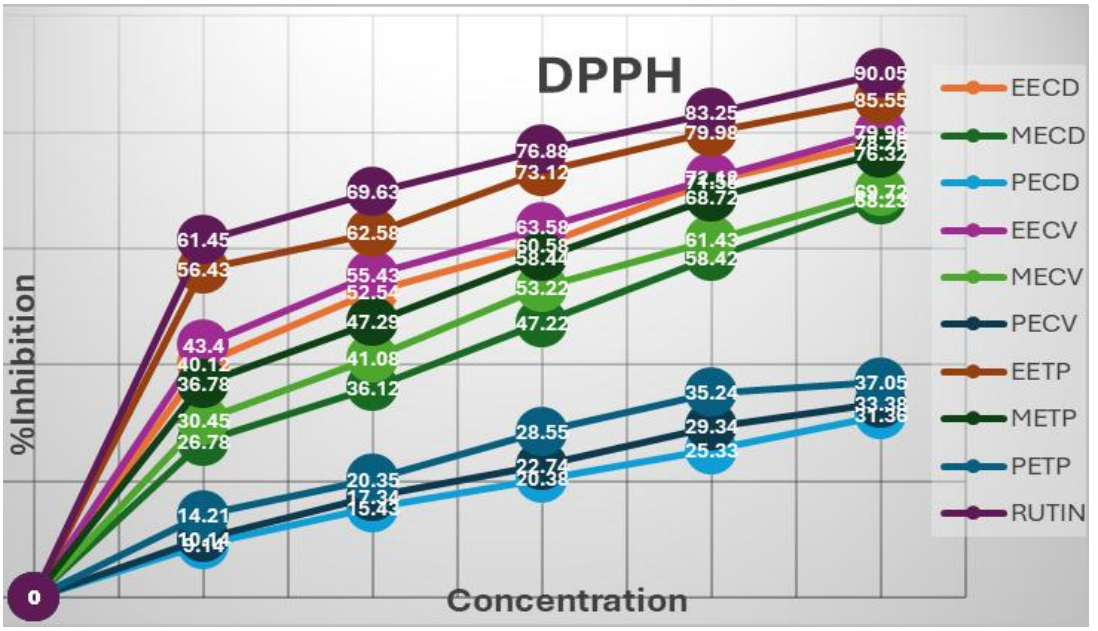

Figure 2: Scavenging potentials of selected extracts in DPPH method

During this reaction of tested plant extract with DPPH, it is converted to suitable form due to capacity in donating hydrogen at a very quick pace (Babili et al., 2022). The level of discoloration reveals the capacity of plant sample in scavenging. IC50 details of ethyl acetate and methanol extract of roots of T. Purpurea was 16.5 and 44.2 µg/ml respectively. IC50 result of ethyl acetate and methanol extract of roots of C. viscosa was 18 and 55.6 µg/ml respectively. The IC50 data of ethyl acetate and methanol extract of our third plant i.e. Cordia dichotoma was 30.5 and 64.3 µg/ml respectively. It was obvious due to previous literature data that all ethyl acetate extracts showing best results due the presence of flavonoids, triterpenoids and other polyphenolic compounds in it.

Scavenging assay with Nitric Oxide

NO is one of the major oxides of nitrogen which is a free radical which is produced from sod. Nitroprusside. Suitable reagent is used. where plant extract having anti-oxidant activity, HNO2 decreases. In our study, 2 mL of 10 mM Na2[Fe(CN)5NO] with phosphate

buffer incubated along with 500 µL of plant extract soln at various concn for 30 minute at

normal room temp. After this incubation period, 500 µL of this soln was added to 1000 µL Griess reagent. The absorbance of this was estimated spectrophotometrically at 546 nanometers (Campelo et al., 2011). This scavenging process was recorded by the below mentioned formula

$$\text{Inhibition Percentage} = 100\frac{\text{ABC} - \text{ABS}}{\text{ABC}}$$

ABC- Absorbance of control; ABS- Absorbance of sample

Different extracts of our selected plants scavenged nitric oxide activity according to concn . All ethyl acetate extracts (EETP, EECV & EECD) have shown IC50 value of 19.5, 21.5 and 30.2 µg/mL respectively whereas METP, MECV & MECD have shown IC50 value 52.4, 70.6, & 84.3 µ.g./mL. Curcumin was taken as standard drug whose IC50 data of 18.5 / mL which was well matched with EETP.

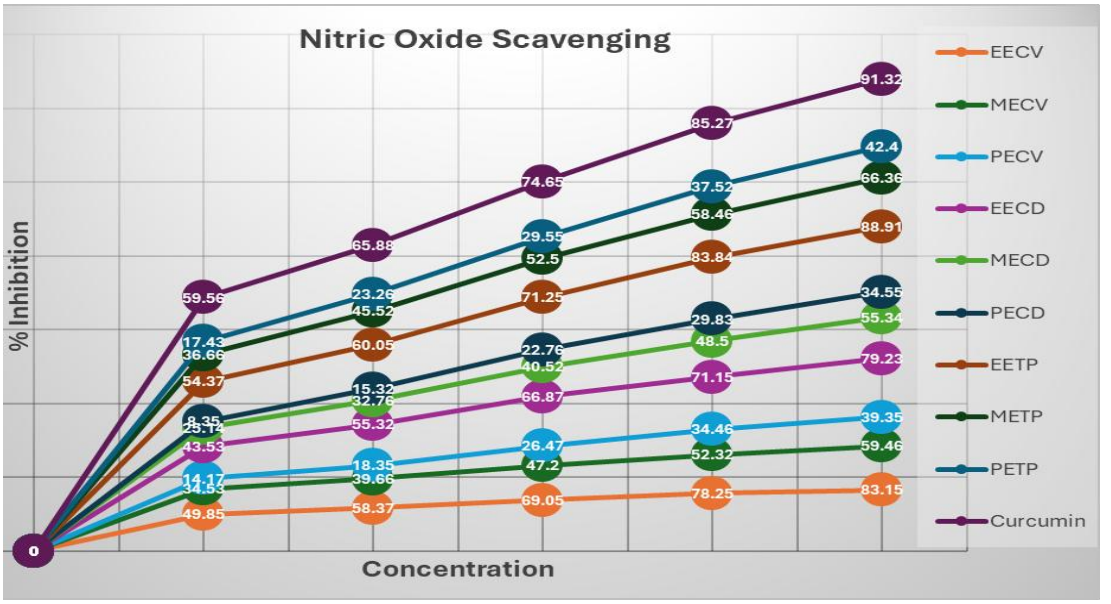

Figure 3: Scavenging potentials of selected extracts in Nitric Oxide test method

Hydroxy Radical Scavenging

Hydroxy radicals (. OH) is one of the highlighted ROS which cause damage in our body. It attaks our cell membrane causing damage even sometimes causing cell death and mutation. In our study the reaction mixture was prepared by adding de-oxyribose at 2,8 milimolar concn , Iron(III) chloride at 100milimolar concn , potassium dihrogenase- pottasium hydroxide buffer at 20 milimolar concn with pH 7.4, EDTA, H2O2 at 1 milimolar concn, vitamin C at 100 mM alongwith extract samples at various concn. This was kept to incubate for 1 hr at room temperature. After which 1 mL each of 2.8 percent C2HCl3O2 and 1% aqueous Tertiary Butyl Alcohol were added to that previous incubated sample which was then heated for 20 minutes at 100 degree centigrade for colour development. The thiobarbituric acid reactive substances found spectrophotometrically at 532 nanometer wavelength in comparison to blank solution.

This scavenging activity was measured by comparison of control absorbance with test extracts (Halliwell et al., 2022).

All our plant extracts reduced degradation process concn wise s shown in figure below. Here all methanolic plant samples (METP & MECV) display IC50 of 90.4 & 99.6 µg/ml respectively. During this study EETP, EECV & EECD resulted very minimum IC50 date (19.7, 20.5 & 40.6 µg/ mL) which can be compared to Curcumin compared to standard drug Curcumin (14.9 µ.g. /mL).

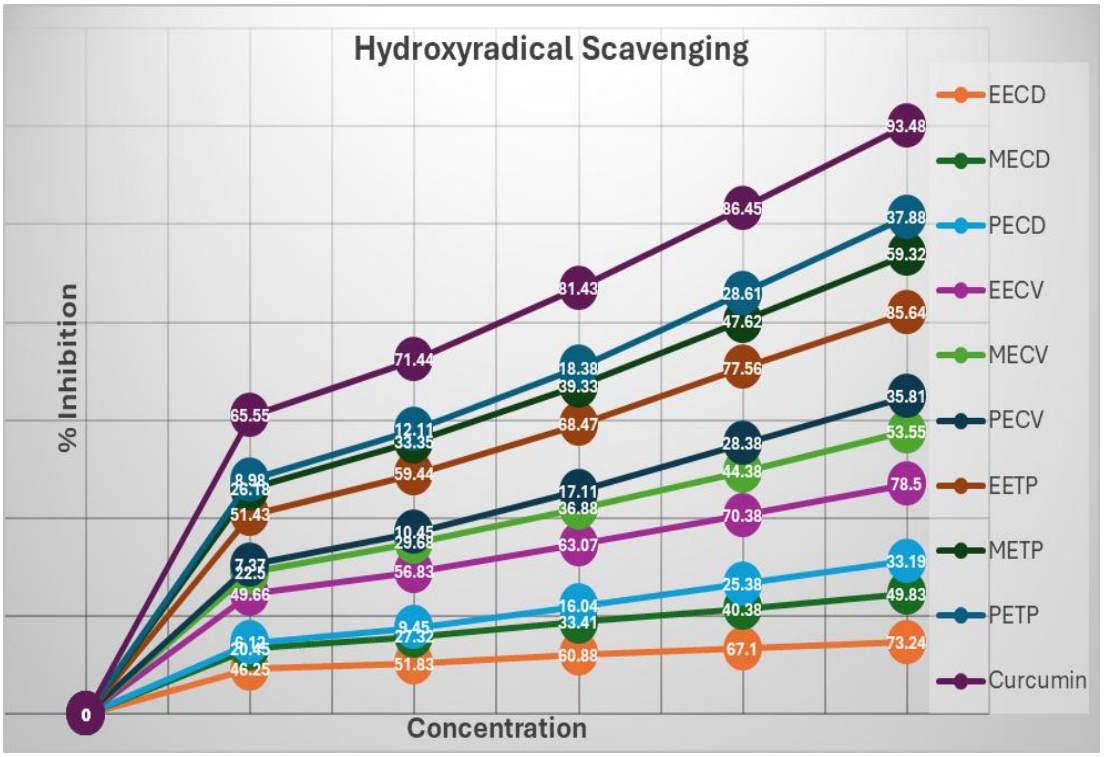

Figure 4: Hydroxy radical Scavenging potentials of selected extracts

Reducing Power Assay

The in vitro antioxidant study was also carried out by reducing power assay method by taking our extracts by Fe3+. To methanol, sample extracts were mixed to which 2500 µL buffer of PO43- & C6N6FeK3 in a test tube which is incubated at fifty-degree centigrade temp. Then 2500 µL 10% Trichloroacetic acid soln mixed, and then centrifugation at 50,000 rpm was done for 10 min. at room temp. From this, 2500 µL sample was taken and absorbance was checked against BHT (Fig 5). During this study methanol extracts (METP, MECV & MECD) showed IC50 values of 65.2, 80.6 & 94.5 µg/ml respectively (Irshad et al., 2012). Reducing activity was better in all ethyl acetate extracts (IC50 Value of EETP, EECV & EACD were 19.7, 20.2, & 25.6 µ.g./mL) when compared to BHA (IC50 =17.5).

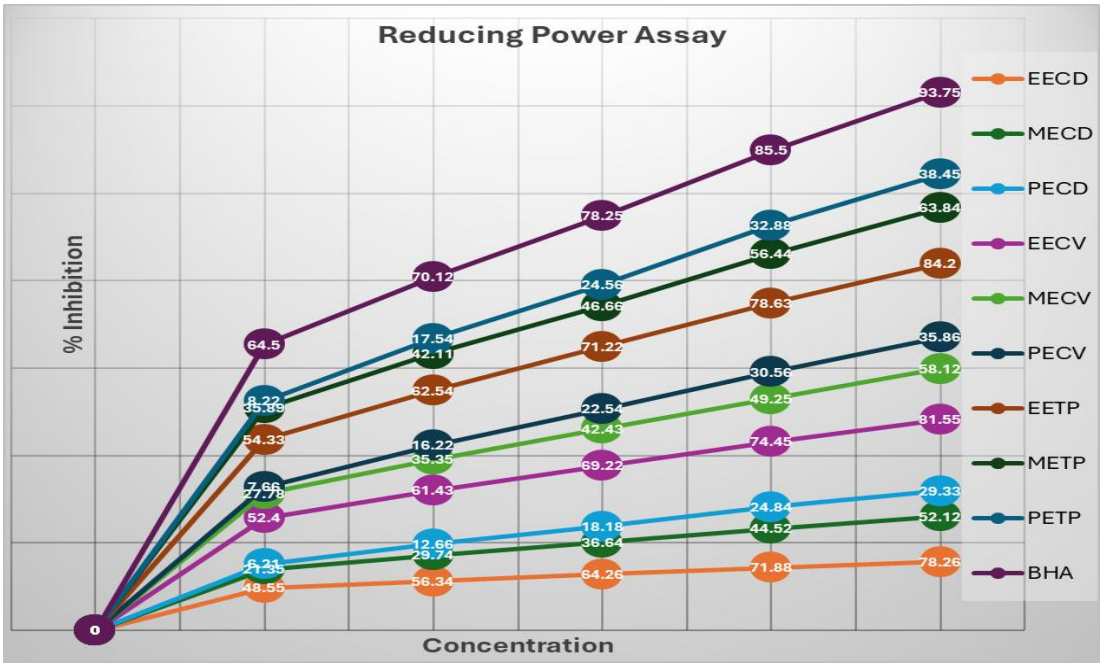

Figure 5: Scavenging potentials of selected extracts in reducing power assay method

Metal chelation assay

This process of chelation of our individual extract towards Fe2+ was estimated by following process mentioned here. 500 µL of individual extract, 1600 µL of De-ionized water and 50 µL of FeCl2 were mixed. 100 µL ferrozine mixed to it after 300 sec. Final mixture incubated and ferrous ion-ferrozine complex abs. was studied spectrophotometrically at 562 nanometer by taking quercetin standard drug (Goodla et al., 2012). This chelation process of extract with ferrous ion is calculated as

$$\text{Inhibition Percentage} = 100\frac{\text{ABC} - \text{ABS}}{\text{ABC}}$$

ABC- Absorbance of control; ABS- Absorbance of sample

From the previous study it is known that ferrozine can form complex with Fe+2.

This process of red-colored complex formation can be controlled by using a chelating sample (i.e. plant extract). This effect of the co-existing chelating agent can be assessed from percentage of reduction of colour. (Figure 6).

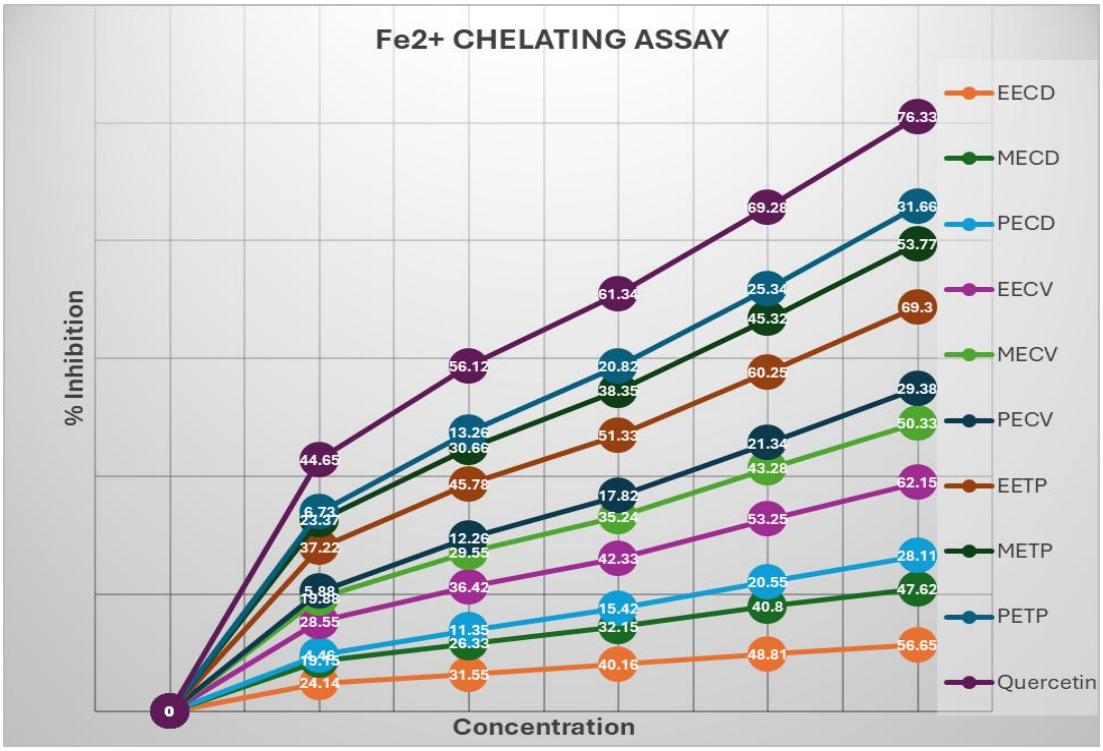

Figure 6: Scavenging potentials of selected extracts in Fe 2+ assay method

2. Conclusion

From literature it has already been revealed that triterpenods & flavonoids present in plants display several biological properties including role as anti-oxidants. All these plants derived anti-oxidants mostly interact with free radicals. In our study, all the ethyl acetate extracts have shown interaction with DPPH and hydroxyl radicals by scavenging it which justifies that some phytoconstituents in these extracts may be responsible for this activity. This scavenging potential and abundant presence of TPC (Total Phenolic Content) of all ethyl acetate extracts justifies anti-oxidant capability.

It has been observed that all ethyl acetate extracts produced best results followed by methanol extract & pet. Ether extract of all our selected plants. The above results prompt us to finalize eligibility of extracts to be utilized in further higher animal-based liver protection ability study.

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