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IN VITRO ANTIOXIDANT EVALUATION OF FLOWRES OF *EHRETIA LAEVIS ROXB* BY DPPH RADICAL SCAVENGING ASSAY AND NIRIC OXIDE RADICAL SCAVENGING ASSAY

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Abstract:

Ehretia laevis, a medicinal plant belonging to the boraginaceae family, is widely recognized for its therapeutic properties in traditional medicine. the present study aimed to explore the phytochemical profile and antioxidant potential of Ehretia laevis through hydro alcoholic extraction and solvent fractionation. The plant material was subjected to hydroalcoholic extraction (70% ethanol), followed by successive fraction into n-hexane, ethyl acetate, and tannins. Anti-oxidant activity was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) and nitic oxide scavenging assays. The hydro alcoholic extract and ethyl acetate fraction demonstated significant free radical scavenging activity, indicating a high antioxidant potential, while the n-hexane and aqueous fraction is particularly enriched with potent antioxidant compounds. The results collectively validate the ethnomedicial relevance of ehretia laevis and provide a basis for its further pharmacological exploration.

Keywords: *EHRETIA LAEVIS, Antioxidant Potential, DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and Nitic oxide Scavenging assays.*

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1.INTRODUCTION[1,2]:

Herbal medicines are a significant part of traditional therapeutic practices. The medicine lists over 2000 natural products, mostly of plant origin. Around 1250 Indian medicinal plants are used in Ayurveda and other traditional formulations for various ailments, including liver disease. Historically, herbal medicines have been used for a long time to treat liver disorders. Approximately 170 plant-derived compounds from 110 plants across 55 families show hepatic-protective properties. Globally around 600 commercial herbal preparations claim activity. The need for effective liver medicines without toxicity drives research into herbal formulations. *Ehretia laevis* has specific botanical characteristics, including its bark, flowers, and fruit (drupe). It has an irregular trunk with a light grey or whitish bark. Flowers are variable in size and shape. They vary from 2 cm to 6.3 cm in length and 1.3 cm to 3.8 cm in width. Flowers of these plants are white in colour. The calyx of these flowers are 2.5 mm long, 3-lobed and the corolla are -8 mm long, in which 5 corolla are lobed. The tube and lobes of corolla are longer than the calyx. Fruits of Ehretia laevis roxb are also known as drupe.

2.PLANT PROFILE[3]:

Ehretia laevis is an Indian medicinal plant.it has deciduous shrub. Ti is considered as smalltree due to its 12 m belongsto family boraginaceae.
Family: boraginaceaea
Habitat: throughout India.
Ayurveda: charmi vrksha
Siddha/Tamil: addula
Folk: kuptaa, datarangi
Binomial name: *Ehretia laevis roxb*

3.CHARACTERISTIC FEATURES[4]:

Ehretia laevis roxb with an irregular trunk. Ehretia laevis has a light grey or whitish bark. Flowers of the plant are usually having variable size and shape. Inconsistently they are 2 cm to 6.3 c in length whereas 1.3 to 3.8 cm in width as dimensions in considered.

4.MEDICINAL BENEFITS[5]:

- A decoction of leaves are indicated in muscular pain also they are prescribed in cough and asthma
- Eczema is treated with tender leaves paste
- Chewing of bark and rubbing on the teeth and lips as an antiseptic
- The roots are used in venereal disease

5.EXPERIMENTAL METHODS[5-7]

PREPARTION OF HYDROALCOHOLIC EXTRACT AND FRACTIONS:

The collected flowers of *Ehretia laevis* Roxb were used for the hydroalcoholic extraction.The lowers were first dried at room temperature for more than fifteen days under the shade; later flowers were pulverized to coarse powder. The powder was subjected to hydro alcoholic extract. Initially the coarse powder of the flower was subjected to 50% of ethanol for about 12 hrs at 50°c. After completion of the process a brownish residue was obtained. The obtained extract was dissolved in distilled water and subjected to liquid-liquid extractionmethod by using solvents different polarity. The process started with n-Hexane, ethyl acetate and aqueous obtain respective fractions. Ultimately all fractions were concentrated by using a rotatory evaporator under reduce pressure.

The extraction procedure was done through hot continuous extraction (soxhlet) method explained as follows.

In this method, the finely ground crude drug is placed in a porous bag or “thimble” madeof strong filter paper, which is placed in chamber E of the soxhlet apparatus. The extracting solvent in flask A is heated, and its vapours condense D. the condensed extractant drips into the thimble containing the C, the liquid contents of chamber E siphon into flask A. this process continuous and is carried out until a drop of solvent from the siphon tube does notleave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and variablewhen converted into a continuous extraction procedure on medium or large scale.

QUALITATIVE PHYTOCHEMICAL SCREENING OF HYDRO ALCOHOLOIC EXTRACT AND ITS FRACTIONS

The extracts obtained were screed to establish the phytochemical profile hence subjected to qualitative phytochemical analyses.

INVITRO ANTIOXIDANT ACTIVITY

Diphenyl picryl hydrazyl (DPPH) radical scavenging assay

Principle:

Antioxidants can react with stable free radical DPPH (1,1-diphenyl 2-picrylhydrazyl).

DPPH radical scavenging gives a strong absorption band at 517 nm with deep violet colour. As this electron becomes paired off this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolourization is stoichiometricWith respect to the number of electrons taken up. The change of absorption produced in this reaction is assessed to evaluate the antioxidant potential to test the samples.

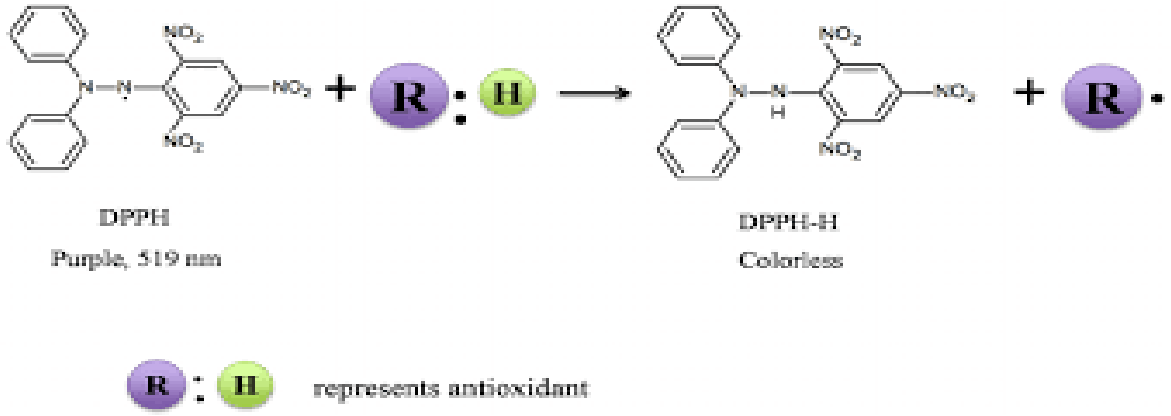

Figure 1: DPPH radical scavenging assay reaction

Materials required:
DPPH solution – 0.3 mg/ml in 0.1mm ethanolic solution
Sample stock solution –500 15.62, 3.25, 62.5, 125, 250, and 500µg/ml in methanol
Standard solution – 1.56, 3.12, 6.25, 12.5, 25, and 50µg/ml of vitamin c in water
Instrument- micro plate reader, biorad (model 550)

Procedure
Extracts 3ml with 6 different concentrations (15.62,31.25, 62.5, 125, 250 & 500 µg/ml) where mixed with 1ml of 0.1 Mm ethanolic solution of DPPH. The absorbance was measured by a spectrophotometer at 517nm at 30 min intervals against a blank pure ethanol). The percentage of radical scavenging activity was calculated. Lower absorbance values show higher free radical scavenging activity. Ascorbic acid was used as a reference standard in different concentration (1.56,3.12,6.25,12.5,25 and 50 µg/ml).The 50% inhibitory concentration value (IC50) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.

Nitric oxide radical scavenging assay
Principal
Sodium nitropruside in aqueous solution at physiological ph. spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ion, which can be estimated by the use of modified Griessilosvay reaction. Nitrite ion react with griess reagent, forming a purple ago due. The ability of the test compound to scavenge nitric oxide is measured in terms of the degree of decrease in the formation of purple azo dye. The absorbance of the chromophore formed measured at 546 nm.

Chemicals and reagents
Sodium nitropruside (5mM), 20mM phosphate-buffered saline (PBS) pH 7.4, Griess reagent (1% sulphanilamide), 2% H3PO4 and 0.1% naphthalethylenediamine dihydrochloride),

Procedure
Preparation of test solution 10mg of extract and it's fractions were dissolved separately and dissolve in 10ml of methanol to obtain solution of 1 mg per ml concentrations.

1.0 ml sodium nitropruside (5mM) in 20mM phosphate-buffered saline(PBS) pH 7.4 was mixed with 1.0ml of different concentrations of test sample and incubated at 25°c for 150 min. The 0.5 ml of the above solution was later reacted with lml of is griess reagent. The absorbance of the chromophore formed during of nitrite with sulphanilamide and subsequent coupling with napthylethylediamine was readed 546nm.

$$\text{NO Scavenger}(\%) = \frac{(\text{Acont} - \text{Atest})}{\text{Acont}} \times 100$$

Where, Acont is absorbance of controlled reaction and a test is the absorbance in the presence of the sample of the extract.

6.RESULTS AND DISCUSSIONS:

Evaluation of hydroalcoholic extract and fractions
The yield of hydroalcoholic extract was measured from Soxhlet extraction apparatus. Total yield collected was about 23.25gm w/w;whereas total extracts n-hexane, ethyl acetate and aqueous fractions obtained by consecutive solvent-solvent extraction of hydroalcoholic extract was about to 0.82, 9.84,61.1% w/w, respectively as shown in table.

Table 1: Description of hydro alcoholic extract yield

Name of extract	Description	polarity	Weight	% yield W/W
Hydroalcoholic extract	Brown	50% ethanol	23.25gm	23.25
N-hexane fraction	Green	100 % n-hexane	1.65gm	0.82
Ethyl acetate	Florescent green	100% ethyl acetate	19.68gm	9.84
Aqueous fraction	Florescent brown	100% Water	30.7gm	61.1

Qualitative phytochemical screening of hydro alcoholic extract, its fraction and sub fractions

The phytochemical extracts were subjected to various chemical tests to prove the presence of phytochemical constituents of interests having various pharmacological activities.

Alkaline Test: Positive tests confirmed presence of flavonoids. Aqueous fractions, ethanolic extracts, ethyl acetate fractions gave positive result.

Dragendorff Test: This test for alkaloids was absent for all tested fractions and extracts

Fehling's Test: Test for carbohydrates was strongly positive for aqueous fractions and ethanolic extracts

Benedict's Test: This test for reducing sugar was positive in aqueous extracts

Keller-killanis Test: This test for glycoside was positive in ethanolic extracts and ethyl acetate fractions

Foam Test: Test for saponin was positive in aqueous extracts and ethanolic extract

Biuret Test: This test for protein was absent in all fractions and extracts

Salkowski Test: Test is divided in terpenoids and sterols. Test for terpenoid was positive in ethanolic extracts, ethyl acetate and n-hexane fractions

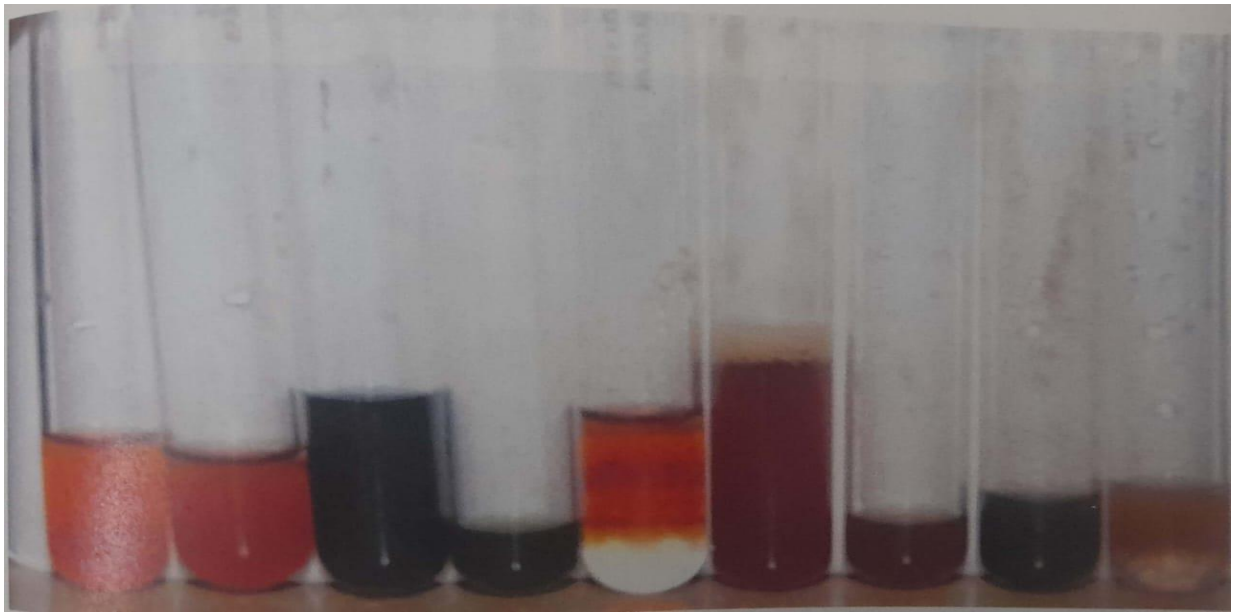

Figure 2: Qualitative phytochemical screening of aqueous fractions

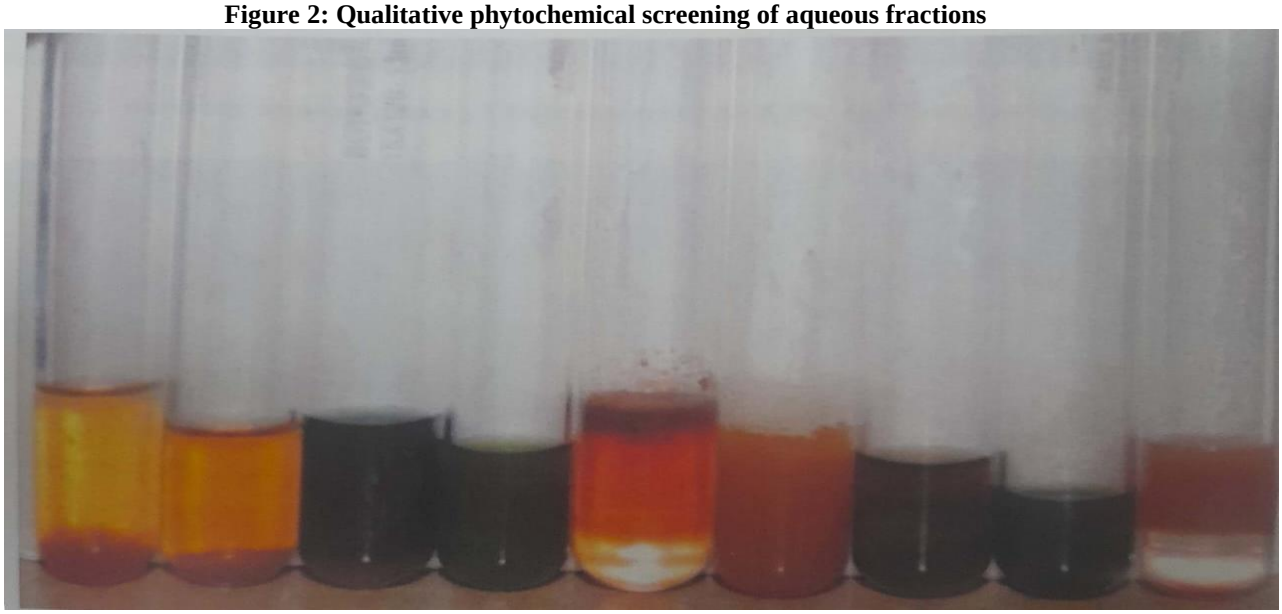

Figure 3: Qualitative Phytochemical Screening Of 50% alcoholic extract

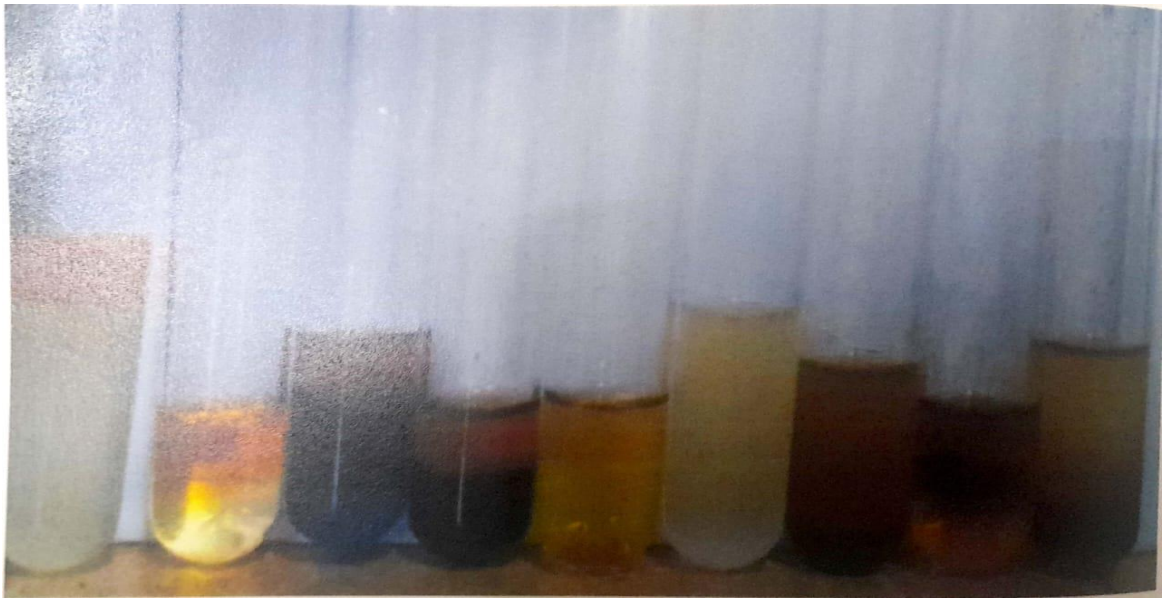

Figure 4: Qualitative phytochemical screening of ethyl acetate fraction

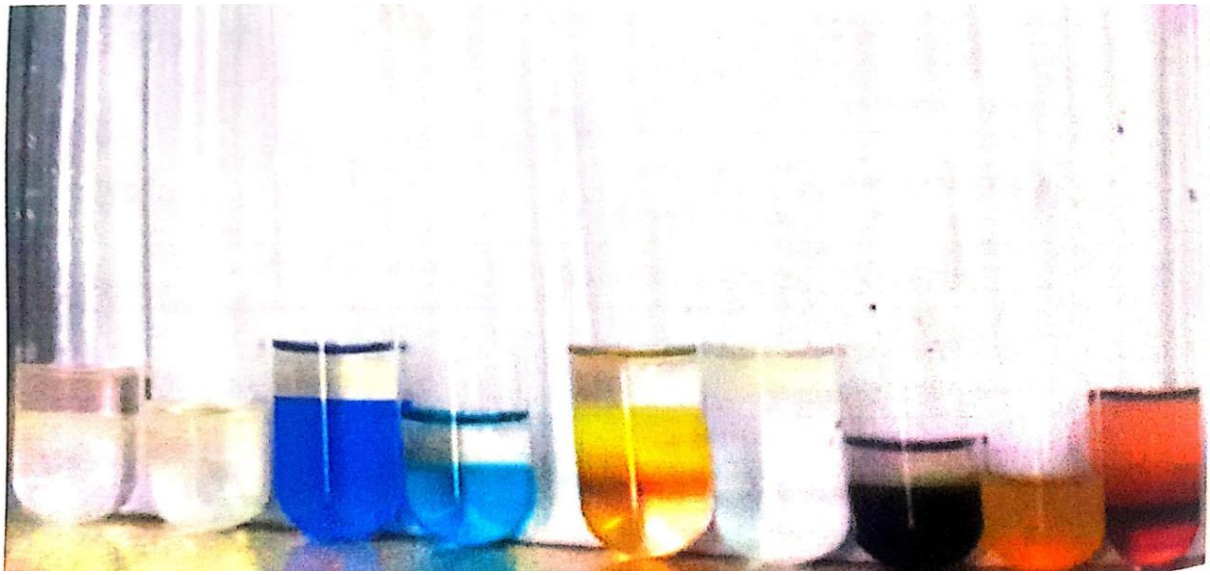

Figure 5: Qualitative Phytochemical Screening of N-hexane fraction

Table 2: Results of phytochemical screening

Sr. no	Test name	Test for	Aqueous Fraction	Ehanolic extract	Ethyl acetate fractions	n-Hexane fraction
1	Alkaline reagent test	Flavonoids	+	+	+	-
2	Dragendroff's test	Alkaloids	-	-	-	-
3	Fehling's test	carbohydrates	++	+	-	-
4	Benedict's test	Reducing sugars	+	-	-	-
5	Keller-killani test	Glycosides	-	++	+	-
6	Foam test	Saponins	++	+	-	-
7	Biuret test	Proteins	-	-	-	-
8	Fecl3	Phenolic compounds	+	+	-	-
9	Salkowski test	Terpenoids	-	+	+	-
		Sterols	-	+	+	+

Note: ++ = strong positive test, + = Weak positive test, - = negative test

***IN VITRO* ANTIOXIDANT ACTIVTY**

Dipheny picryl hydrazyl (DPPH) radical scavenging assay

The antioxidant potential of aqueous fraction, hydro alcoholic and ethyl acetate fraction was evaluated by DPPH method. In free radical scavenging activity, DPPH accepts an electron or hydrogen radical to become stable diamagnetic molecule. The efficacy of antioxidant is measured by their capability to converting stable DPPH to yellow colored biphenyl picryl hydrazine. As the action of antioxidant increases, there is decrease in absorbance of DPPH radical at 517nm. This occurs because the antioxidants scavenge the radicals by donating hydrogen. Among the three extracts, the hydroalcoholic extract of *Ehretia laevis* showed maximum antioxidant potential with an IC50value of 56.50µg/ml followed by aqueous fractions (IC50239.72µg/ml) and ethyl acetate fraction (IC50350.85µg).

Table 3: DPPH Radical scavenging assay

Time	Aq.Fraction Mg/ml	50% ethanolic µg/ml	Ethyl acetate µg/ml	Ascorbic acid µg/ml
15	29.09	18.18	8.91	7.3
30	34.55	34.55	9.18	34.5
60	40.18	58.36	22.09	47.3
120	42.82	79.18	36.36	61.8
240	51.27	87.64	40.36	70.9
480	66.64	91.18	60.18	92.5
IC50	**239.7260274**	**56.50793651**	**56.50793651**	**7.777778**

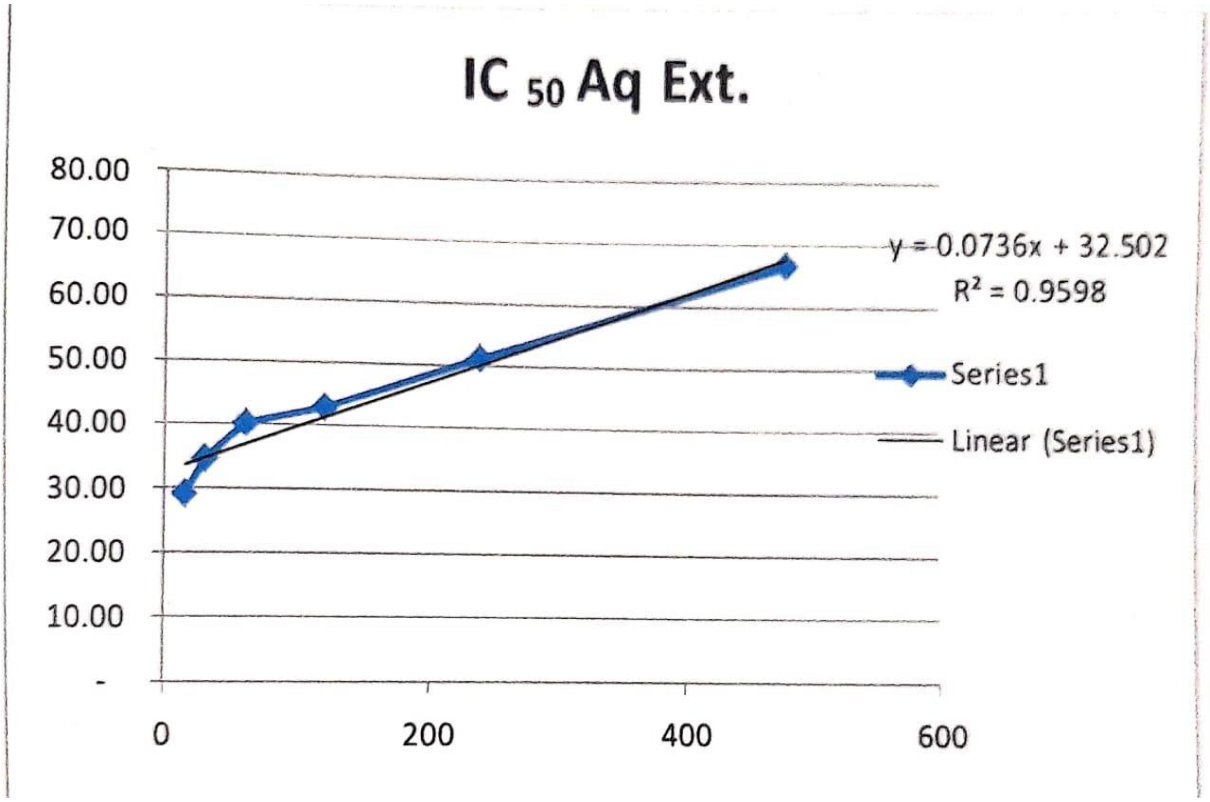

Figure 6: Linearity graph of scavenging action of DPPH on aqueous extract of ehretia laevis roxb

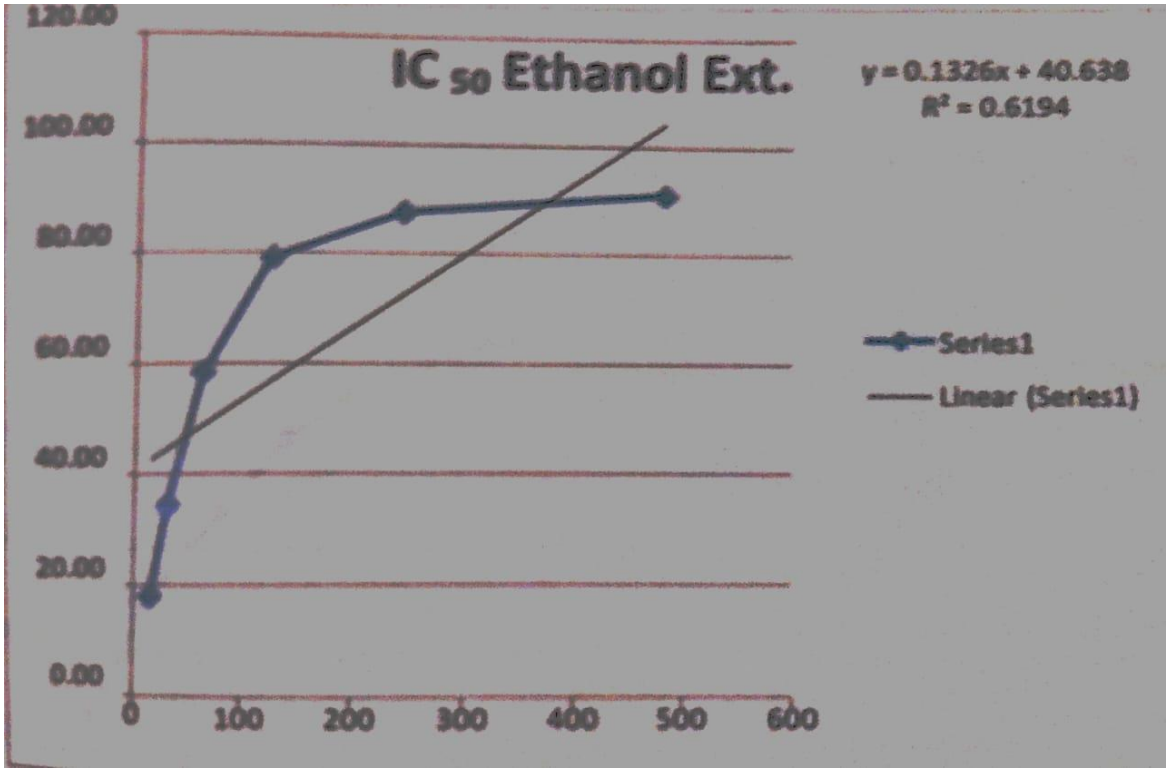

Figure 7: Linearity graph of scavengoing action of DPPH on 50% ethanolic extract ehretia laevis roxb

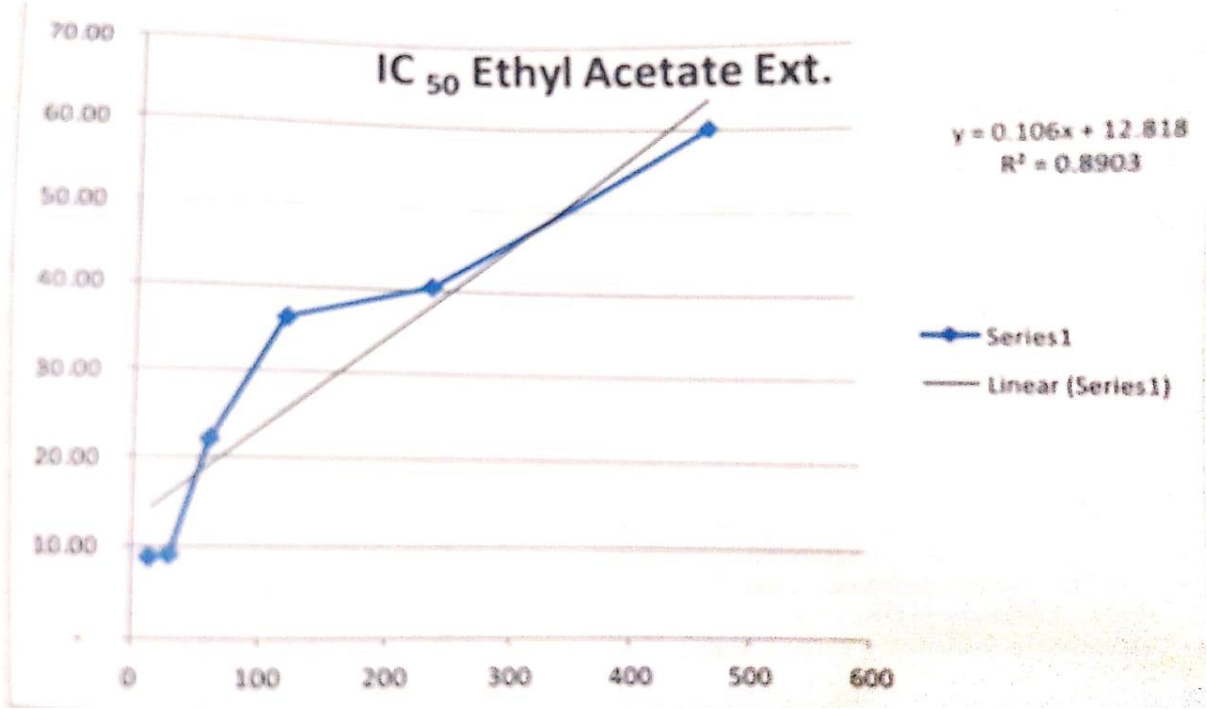

Figure 8: Linearity graph of scavenging action of DPPH on ethyl acetate extract of ehretia laevis roxb

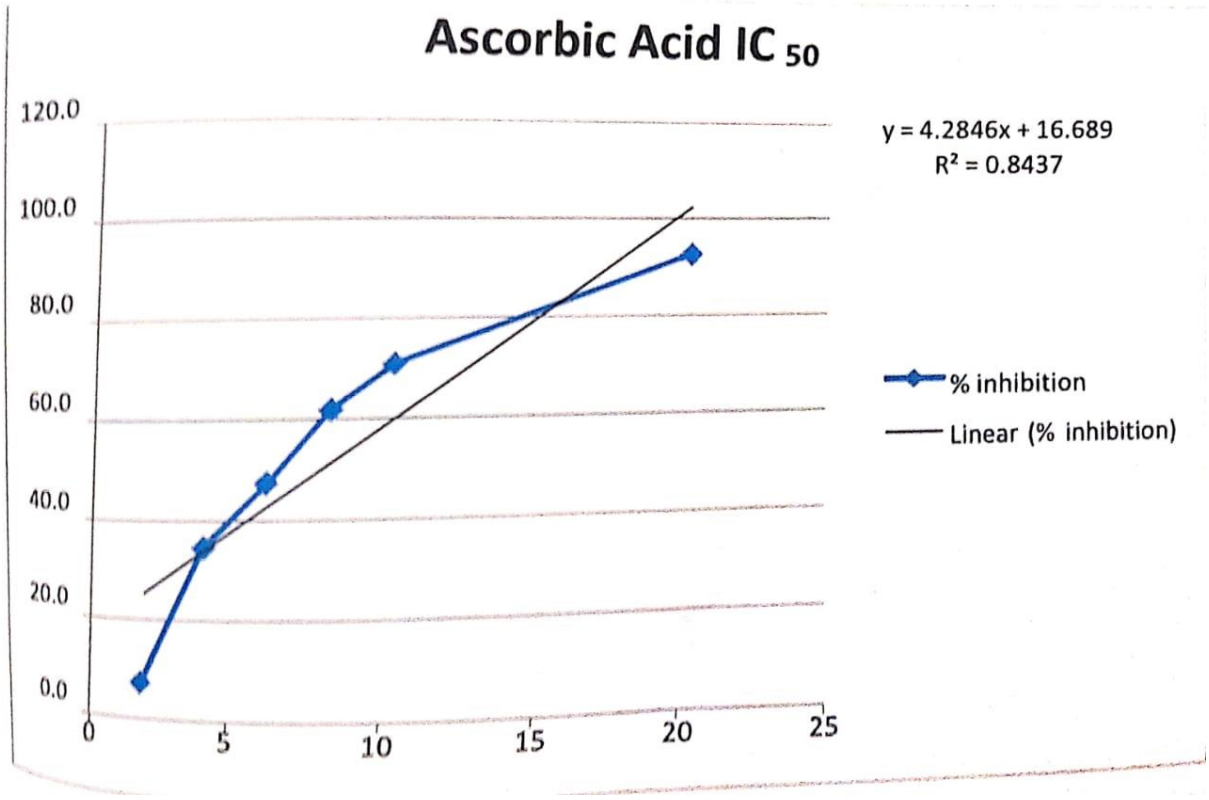

Figure 9:Linearity graph of scavenging action of DPPH of Ascorbic acid

7.CONCLUSION:

The antioxidant potential of aqueous fraction, hydro alcoholic and ethyl acetate fraction was evaluated by nitric oxide radical scavenging assay method. Among the three extracts, the hydroalcoholic extract of ehretia laevis showed maximum antioxidant with an IC50 value of 478.76 µg/ml followed by aqueous fraction (IC50 659.87 µg /ml)

Table 4: DPPH and Nitric oxide radical scavenging assay

Assay	50% ethanolic µg/ml	Ethyl acetate µg/ml	Aq. Fraction µg/ml	Ascorbic acid	Quercetin
DPPH	**56.50**	350.85	239.72	7.77	-
NITIC OXIDE	**478.76**	892.43	659.87	-	33.68

Among all the tested hydro alcoholic extract showed a highest antioxidant potential by both DPPH And NIRIC OXIDE radical scavenging assay.

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