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Phytochemical Screening and Comparative Study of Antioxidant Activity of Different Parts of Mulberry Plant

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ABSTRACT

Mulberry is commonly used for silkworm rearing due to the presence of chemo-factors such as morin and sitosterol in the leaves. Mulberries are important in containing free radicals and pro- oxidants produced in the body as a result of metabolism and phagocytosis. Pharmacological uses of mulberry include phytochemical composition, antioxidant and medicinal properties. The pharmacological activity of mulberry plants can be predicted by the identification of phytochemicals. Although various modern techniques are used to determine phytochemicals, qualitative tests are still used for preliminary phytochemical screening of plants. Alkaloids, carbohydrates, proteins, flavonoids, phenolic compounds, saponins, tannins, quinones, anthraquinones, and other phytochemicals. The purpose of this research was to determine the antioxidant activity of the mulberry plant's fruits, stems, and leaves. The presence of antioxidant properties in the extract of mulberry plant was confirmed using the DPPH method.

Keywords: Phytochemical Screening, Antioxidant Activity, Mulberry, DPPH.

I. INTRODUCTION

Mulberry is a member of the Moraceae family and belongs to the genus Morus. It contains 24 different species, including black mulberry (M. nigra), red mulberry (M. rubra), and white mulberry (M. alba), among others [1]. Black mulberry, like red mulberry, has a strong flavour. When ripe, the fruit is almost black and 2.5 cm (1 inch) in diameter. Mulberry fruits colour derived from anthocyanins [2]. Mulberries are a rich source of various vitamins and minerals it contains an excessive amount of anthocyanin and it is used as food [3]. The extracts of different parts of plant such as fruits, leaves and bark exhibit diverse pharmacological activities such as antioxidant, immunomodulatory, anti-inflammatory, anticancer, antifungal, antimicrobial, antidepressant, anthelmintic, anxiolytic, hepatoprotective, and

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cardioprotective activities as depicted in figure no. 1[4].

Antioxidant activity is measured by efficiency of an atom that scavenges free radicals by accepting or donating free radial that results into the oxidation of substrate. Free radicals are molecules, ions or atom characterized by presence of unpaired electron and are highly active to chemical reactions which builds oxidative stress derived from imbalance between the production and elimination of free radicals.

Free radicals are usually oxygen, nitrogen and sulphur moieties. These free radical moieties are group of molecules known as reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl^-$) and the superoxide radical anion(O_2^-) reactive sulphur species (RSS) and reactive nitrogen species (RNS) [5]. These free radicals are produced during metabolic processes and different functional activities, which plays a significant role in cell signalling, gene expression, apoptosis and ion transportation [10]. Normally, cells defend themselves against free radicals by employing intracellular enzymes that maintains free radical concentration at low level [11]. Whereas, increased concentration of free radical attacks side chains of amino acid in proteins, bases in nucleic acid and fatty acids which causes oxidative stress results into damaging of DNA, RNA, Lipids and Proteins that eventually contributes in the pathogenesis of variety of diseases such as cancer, autism, aging, diabetes, cataract, cardiovascular disease and Alzheimer's disease [6-9].

In treatment to cure or decrease ROS induced oxidative stress, the human body has its own defence system which includes metal chelating and free radical scavenging activity. But in actual, it is insufficient, which leads to different pathological diseases [10]. In addition of this intake of food derived substance may help to maintain an antioxidant activity.

Antioxidants are the molecules that directly react with free reactive radicals and destroy them by accepting or donating electron to eliminate free radicals. Sources of antioxidants include tannins, flavonoids, vitamins, polyphenols, carotenoids, and lutein [11].

Methods used to determine antioxidant potential

Electron transfer methods (ET)

- DPPH free radical scavenging (DPPH)
- Ferric reducing antioxidant power (FRAP)
- Trolox equivalent antioxidant activity (TEAC)
- Total flavonoid content
- Total phenolic content [12]

From amongst method, we have selected DPPH method for determination of antioxidant activity.

Figure 1: Fruits, Leaves and Stems of M. nigra plant

II. EXPERIMENTAL WORK

A. Preparation of sample

Whole parts of selected M. nigra plant was collected from tropical region of western ghat of Maharashtra in summer season and samples were separated into leaves, stem and fruits. Samples washed three times with distilled water. To preserve the phytochemical constituents, present in them, the samples was

allowed to dry under shade keeping over the newspaper. Then the half-dried parts were cut into small pieces by using stainless steel knife and kept beneath shadow for drying. About 10 days are required for drying samples completely. Dried parts of plant were stored in air tight container for further processing.

Grinding or powdering

The stored dried plant parts from container were pulverized into coarse powder by means of manual blender. Formed powdered plant material was store in air tight container for further process. The weight of powder from dried plant parts of leaves, fruits and stem was found to be 242g, 178g and 116g respectively. The aim of grinding the plant materials is to rupture its tissue and cell structure so that the medicinal constituents are exposed for extraction solvent [13].

Figure 2: Prepared powder samples of Fruits, Leaves and Stem of M. nigra plant

B. Preparation of extract

Extraction is a process which involves the separation of medicinal compound from reservoirs of plant materials by using specific solvent and employing standard procedure. Amongst the all-reported methods, we selected Soxhlet extraction, maceration method for alcoholic extract and ultrasonication method for aqueous extract.

1. Preparation of alcoholic extract by using Soxhlet apparatus

Soxhlet extraction is a popular method for extracting phytochemicals from solid plant materials. The Soxhlet extraction technique is still used to compare the performance of modern extraction techniques [14]. Soxhlet extraction is a very useful tool for preparative purposes in which the analyte is concentrated as a whole from the matrix or separated from specific interfering substances. Solvent extraction of solid samples, also referred to as solid-liquid extraction [15].

Procedure

- Reserved powdered samples of leaves, fruits, and stem was taken and weighed about 20 gm and placed inside a thimble made from thick filter paper.
- This is filled into the main chamber of the Soxhlet extractor.
- Ratio of 1:10 i.e., powder: solvent was followed. This Soxhlet extractor is placed in to a flask containing the extraction solvent.
- The Soxhlet is then equipped with a condenser. Then added 200 ml ethanol. The solvent is heated at 60°C. The solvent vapor rises up a distillation arm and floods into the chamber containing the solid thimble. The condenser ensures that any solvent vapor cools and drips back into the solid material chamber.
- The warm solvent gradually fills the chamber containing the solid material. In the warm solvent, some of the desired compound will dissolve.
- When the Soxhlet chamber is nearly full, the syphon side arm automatically empties the chamber, with the solvent running back down to the distillation flask. This cycle can be repeated many times over the course of hours or days.
- A portion of the compound dissolves in the solvent during each cycle, and the desired

compound is concentrated in the distillation flask after each cycle. The benefits of this system are that instead of passing many portions of worm solvent through the sample, only one batch of solvent is recycled.

- Following extraction, the solvent is typically removed using a rotary evaporator, yielding a portion of the extracted compound. The non-soluble portion of the extracted solid is usually discarded in the thimble [16].

2. Preparation of alcoholic extract by using Maceration method

Procedure

- Taken three beakers (250 ml each), wash & clean it properly. Ratio of 1:10 is followed for extraction.
- Taken 200 ml of ethanol in each beaker and mixed with 20 g of powdered samples. Stick the label on each beaker with numbering i. e. 1) Fruit, 2) Stem, 3) Leaves.
- Add 20g fruits powder in first beaker, 20g stem powder in second beaker & in third beaker 20g leaves powder.
- Mix it properly with the help of glass stirrer. Close all three beakers with the help of aluminium foil. Kept it for 7-8 days.
- After 8 days filter the mixture, extract is received.

Figure 3: Maceration extract of leaves, fruits and stem sample.

3. Preparation of aqueous extract by using Ultrasonication method

Multiple transducers are typically mounted beneath a stainless-steel tank, which serves as the ultrasound source in an ultrasound bath. Most commercial ultrasonic baths produce adequate ultrasound levels for washing, degassing solvents, and removing adsorbed metals and organic contaminants from environmental samples, but they are less efficient for extracting matrix bound analytes [17]. The strength should be high enough to cause cavitations within the bath extraction vessel; this is uncommon in traditional ultrasonic baths [18]. The location of the vessel within the bath is an important factor in determining extraction performance. For a bath with a single transducer at the bottom, the extraction vessel should be placed just above the transducer, as power distribution would be optimal at this location [19].

Procedure

- Take three beakers (250 ml each), wash & clean it properly. Ratio of 1:20 is followed for extraction.
- Take 100 ml water in each beaker. Add 5g fruits powder in first beaker, 5g stem powder in second beaker & in third beaker 5g leaves powder.
- Mix it properly with the help of glass stirrer. Sticks the label on each beaker with numbering i. e. 1) Fruit, 2) Stem, 3) Leaves. Close all three beakers with the help of aluminium foil.
- Kept it in ultrasonic bath for 1 h at 50°C. After 1 h filter the mixture, extract is received.

C. Phytochemical screening

The medicinal plants contain some important types of biologically active constituents called phytochemicals which are of great importance and exhibit potency towards the pharmacological activity which is done by using specific biochemical test. Phytochemical screening confirmed the presence of Phyto-constituents like alkaloids, flavonoids, glycosides, phenols, lignin, saponins,

sterols, tannins, anthraquinone, and reducing sugar. Their confirmations, identifications, and characterization are very important, and to detect these phytochemicals, a standard procedure previously reported which is represented as follows [20].

Test for Alkaloids (Dragendroff's Test & Picric acid Test)

- Taken 1ml of filtrate, added 2ml of Dragendroff's reagent. Presence of reddish-brown precipitate indicates alkaloids presence [21].
- To 1ml filtrate, added 3-4 drops of 2% picric acid solution. Formation of orange colour indicates alkaloids presence [22].

Test for carbohydrates (Barfoed's Test)

- To 1ml of filtrate, added 1ml Barfoed's reagent then heat the solution for 2 min. Presence of red precipitate indicates carbohydrates presence [23].

Test for Reducing Sugars (Fehling's Test)

- Taken 1ml Fehling's solution A & 1ml Fehling's solution B, added 1ml of extract then boiled in water bath. Formation of precipitate indicates reducing sugars present [24].

Test for Glycosides (Aqueous NaOH Test)

- To the 1ml of extract dissolve in 1ml of water then added few drops of aqueous NaOH solution. Presence of yellow colour indicates glycosides present [25].

Test for Cardiac Glycosides (Bromine Water Test)

- Taken 1ml of extract in 2ml of bromine water. Formation of yellow precipitate indicates cardiac glycosides presence [26].

Test for Proteins & Amino Acids (Millon's Test)

- Taken 2ml of extract, added 3-4 drops of Millon's reagent. Formation of white precipitate indicates proteins or amino acids presence [27].

Test for Flavonoids (Lead Acetate Test)

- To 1ml of plant extract, added 1ml of 10% lead acetate solution. Formation of yellow precipitate indicates flavonoids presence [28].

Test for Phenolic Compounds (Iodine Test)

- To 1ml extract, added few drops of dil. Iodine solution. Presence of transient red colour indicates phenolic compound presence [29].

Test for Tannins (Braymer's Test)

- Taken 1ml of filtrate in 3ml of distilled water then added 3 drops of 10% Ferric chloride solution. Presence of blue-green colour indicates tannins present [30].

Test for Phytosterols (Sulphur Test)

- Taken 2ml of extract & added pinch of sulphur powder. Sulphur powder sinks to the bottom indicates phytosterols are presence [31].

Test for Terpenoids

- Taken 2ml of chloroform in 5ml of plant extract, added 3ml of conc. H_2SO_4. (Boiled on water bath). Presence of grey coloured solution indicates terpenoids presence [32].

Test for Triterpenoids (Salkowski's Test)

- Taken 2ml of filtrate & added few drops of conc. H_2SO_4. (Shaken well and allowed to stand). Presence of golden yellow layer (at the bottom) indicates triterpenoids presence [33].

Test for Quinones (conc. HCl Test)

- Taken 1ml extract in conc. HCl. Presence of green colour indicates quinines are present [34].

Test for Anthraquinones (Bontrager's Test)

- To 10 ml 10% ammonia solution added in extract (Shaken vigorously for 30 sec). Formation of pink, violet or red colour solution indicates anthraquinones presence [35].

Test for Anthocyanins (HCl Test)

- Taken 2ml of plant extract in 2ml 2N HCl (few ml of ammonia). Indicate pink-red solution which turns blue-violet after addition of ammonia then anthocyanins presence [36,37].

Test for Resins (Turbidity Test)

- Taken 2ml of extract in 4ml of 4% HCl. Formation of turbidity indicates resins are presence [38].

D. Determination of moisture content

The crude drugs show presence of moisture content which can be a crucial factor in deterioration of shelf life of that drug. Hence it is necessary to calculate the presence of moisture content.

- Dry the empty dish with lid in oven at 105^{0}C for 3 hours and transfer to desiccator to cool.
- Weigh the empty dish with lid.
- Moisture content can be determined by heating the crude drug at 105^{0}C in oven to a constant weight and calculating the loss of weight [39].
- Percentage moisture content can be calculated by using formula-

% Moisture content = weight of water in material/oven dry weight of material * 100

E. Determination of titratable acidity

Titratable acidity or acid value corresponds to the total concentration of titratable acids in a sample. Titratable acidity (TA) is an important characteristic of the quality of numerous products such as vegetable oils, juices, wines, petroleum, motor oils, polyesteric resins, plasticizers and others.

- Titratable acidity was determined according to the AOAC official method 942.15.
- 5 g of sample diluted in 25 ml of distilled water and titrated by 0.1 N sodium hydroxide (NaOH) to pH 8.1 [40].
- The titratable acidity was expressed as g. citric acid/Kg of sample.
- According to the following equation:

Titratable acidity = (V*0.1*1000*0.064)/m

Where, 0.1 is the normality of NaOH (N)

0.064 is the conversion factor for citric acid

V is the volume of NaOH required (ml)

m is the mass of sample used.

F. Determination of antioxidant activity

From all the listed methods we have selected DPPH assay method for the determination of antioxidant activity. This was carried according to Blois method with a slight modification. This assay is based on the ability of the antioxidant to scavenge the radical cation DPPH. Chemically DPPH ($C_{18}H_{12}N_5O_6$; Mol wt. 394.33) is a 2,2-Diphenyl-1-picrylhydrazyl a stable free radical. On mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet colour. DPPH radical is reduced by the donor molecule from plant extract. The DPPH molecule is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by constituents from plant extract. The reaction is accompanied by changing the DPPH colour measured at 517 nm, and discolouration acts as an indicator of antioxidant activity [41-43].

Procedure

1. DPPH stock solution of concentration 0.004% was used by dissolving 4 mg of DPPH into 100 ml of ethanol.
2. Different concentrations of plant extracts of fruit, stem and leaves was prepared using 20, 40, 60, 80, 100 and 200 μg/ml by taking 20, 40, 60, 80, 100 and 200 μl and making volume up to 3 ml by using ethanol.
3. Then added 1 ml of DPPH stock solution and incubated for 30 min followed by measuring absorbance at 517 nm.
4. Solvent used for extraction was considered as a blank. While, ethanol along with DPPH solution (3 ml + 1 ml) was considered as a control for further measurement.
5. BHT (Butylated hydroxy toluene) of same i. e., 20, 40, 60, 80, 100 and 200 μg/ml concentration was taken as standard for comparative measurement study. Stock solution prepared by dissolving 0.1 g of BHT in 10 ml of ethanol.
6. After 30 min, absorbance was measured at 517 nm against a blank containing all reagents except the test samples.
7. The percentage of inhibition of DPPH (I%) was calculated using the following equation:

% Antioxidant activity = ab(control) – ab(sample)/ab(control) *100

The IC_{50}, the concentration giving 50% inhibition of DPPH, was read off a graph of I% (percentage inhibition) versus extract concentration [44].

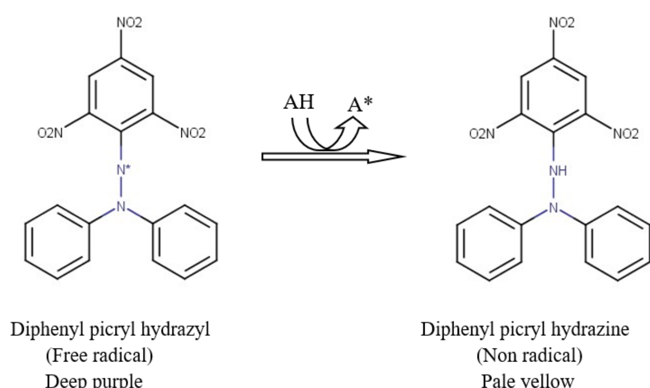

Figure 4 : Reaction involved in DPPH assay

III. RESULT AND DISCUSSION

A. Phytochemical screening

The phytochemical screening of crude ethanolic and aqueous extracts prepared from different extraction methods of leaf, stem and fruit samples of Mulberry plant was carried out. Screening of methanolic extract prepared by Soxhlet extraction studies reveals the presence of alkaloids, carbohydrates, reducing sugars, cardiac glycosides, phenolic compounds, flavonoids, tannins and resins in fruit and stem extract with specifically absence of carbohydrates in stem extract. Whereas, leave extract shows only presence of flavonoids, quinones and tannins as shown in table 1

Table 1 Phytochemical screening of ethanolic extract prepared by Soxhlet extraction of fruit, leaf and stem sample

Phytochemical constituent	Test	Interferences		
		Fruits	Stem	Leaves
Alkaloids	Dragendroff's test	-	-	-
	Picric acid test	+	+	-
Carbohydrates	Barfoed's test	+	-	-
Reducing sugar	Fehling's test	+	+	-
Cardiac glycosides	Bromine water test	+	+	-
Protein and amino acids	Millon's test	-	-	-
Phenolic compounds	Iodine test	+	+	-
Flavonoids	Lead acetate test	+	+	+
Tannins	Braymer's test	+	+	+
Terpenoids	Chloroform + sulphuric acid test	-	-	-
Triterpenoids	Salkowski' test	-	-	-
Quinones	Conc. HCl test	-	-	+
Anthraquinones	Bontrager's test	-	-	-
Anthocyanins	Conc. HCl test	-	-	-
Resins	Turbidity test	+	-	-
Gums and mucilage	Alcohol test	-	-	+

Screening of methanolic extract prepared by maceration method studies reveals the presence of alkaloids, reducing sugars, phenolic compounds, flavonoids, anthraquinones and anthocyanins in fruit extract. While, stem extract shows presence of phenolic compounds and flavonoids in stem extract and leave extract shows only presence of phenolic compounds gums, mucilage and flavonoids as shown in table 2

Table 2: Phytochemical screening of ethanolic extract prepared by Maceration method of fruit, leaf and stem sample

Phytochemical constituent	Test	Interferences		
		Fruits	Stem	Leaves
Alkaloids	Dragendroff's test	+	-	-
	Picric acid test	+	+	-
Carbohydrates	Barfoed's test	-	-	-
Reducing sugar	Fehling's test	+	-	-
Cardiac glycosides	Bromine water test	-	-	-
Protein and amino acids	Millon's test	-	-	-
Phenolic compounds	Iodine test	+	+	+
Flavonoids	Lead acetate test	+	+	+
Tannins	Braymer's test	-	-	-
Terpenoids	Chloroform + sulphuric acid test	-	-	-
Triterpenoids	Salkowski' test	-	-	-
Quinones	Conc. HCl test	-	-	-
Anthraquinones	Bontrager's test	+	-	-
Anthocyanins	Conc. HCl test	+	+	-
Gums and mucilage	Alcohol test	-	-	+

Screening of aqueous extract prepared by ultrasonication studies reveals the presence of alkaloids, reducing sugars, phenolic compounds, flavonoids, and anthocyanins in fruit extract. With specifically presence of flavonoids and anthocyanins in leave extract. Whereas, stem extract shows only presence of cardiac glycosides, flavonoids, phenolic compounds, gums and mucilage as shown in table 3

Table 3 Phytochemical screening of aqueous extract prepared by Ultrasonication of fruit, leaf and stem sample

Phytochemical constituent	Test	Interferences		
		Fruits	Stem	Leaves
Alkaloids	Dragendroff's test	+	-	-
	Picric acid test	+	-	-
Carbohydrates	Barfoed's test	-	-	-
Reducing sugar	Fehling's test	+	-	-
Cardiac glycosides	Bromine water test	-	-	+
Protein and amino acids	Millon's test	-	-	-
Phenolic compounds	Iodine test	+	+	+
Flavonoids	Lead acetate test	+	+	+
Tannins	Braymer's test	-	-	-
Terpenoids	Chloroform + sulphuric acid test	-	-	-
Triterpenoids	Salkowski' test	-	-	-
Quinones	Conc. HCl test	-	-	-
Anthraquinones	Bontrager's test	-	-	-
Anthocyanins	Conc. HCl test	-	+	-
Gums and mucilage	Alcohol test	-	+	+

B. Determination of moisture content

The moisture content of crude drugs is often an important criterion of their quality, if the moisture content exceeds a certain value decomposition of active principles may occur and microbial growth may take place. Determination of moisture content was carried out for stem, bark and fruit sample of mulberry plant and results are obtained as shown in table 4

Table 4 observed % moisture content of fruit, leaf and stem sample

Sr. No.	Sample	% Moisture content
1	Fruit	16.95
2	Stem	11.73
3	Bark	11.11

When we compare observed data amongst the result of percentage moisture content it reveals higher moisture content in fruit as compared to stem and bark sample of mulberry plant.

C. Determination of titratable acidity

The titratable acidity of the sample was found to be significantly different for each sample. Initially burette reading in triplicate was taken and titratable

acidity was calculated by given formula and the results are shown in below table 5

Table 5 observed titratable acidity of fruit, leaf and stem sample

Sr. No.	Sample	Burette reading (ml)	Titratable acidity
1	Fruit	4	5.12
2	Stem	1	1.28
3	Bark	0.8	1.02

D. Determination of Antioxidant activity

The antioxidant activity of Soxhlet, Maceration and Ultrasonication extracts from the fruit, leaf and stem samples of M. nigra at different concentrations (20, 40, 60, 80, 100 and 200) was carried out. Soxhlet extraction showed activity ranging from 59-69 % for fruit sample, 41-64 % for stem sample and 32-45% for leaf sample. The results are expressed in below table 6

Table 6 observed % Antioxidant activity of Soxhlet extraction of fruit, leaf and stem sample

Sr. No.	Concentration	% Antioxidant Activity		
		Fruit	Leaf	Stem
1	20	60.44	45.14	64.55
2	40	59.77	39.92	49.92
3	60	69.02	37.61	59.17
4	80	60.44	32.46	42.83
5	100	59.17	33.20	41.56
6	200	57.31	36.86	62.68

The absorbance was gradually increased with increasing concentrations at certain concentration and then decreases or remains same gradually over increasing concentration of Soxhlet extracts. Higher antioxidant activity is seen at 60, 20 and 20 µg/ml conc. whereas, low activity is seen at 200, 80 and 100 µg/ml conc. of fruit. Leaf and stem sample respectively. The antioxidant activity of different Soxhlet extracts as equivalent to DPPH was in the order of fruit > stem > leaf sample of M. nigra.

The results reveal that antioxidant activity is higher in fruits and comparatively low in leaves of M. nigra. The graphic representation of comparison of % antioxidant activity of Soxhlet extract of fruit, leaf and stem sample was shown in below figure 5

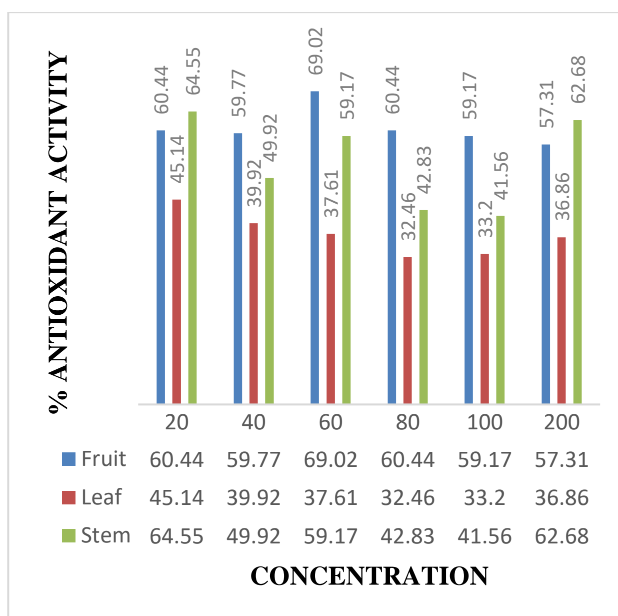

Figure 5 Comparison of % Antioxidant activity of Soxhlet extraction of fruit, leaf and stem sample

The antioxidant activity of Maceration extracts from the fruit, leaf and stem samples of M. nigra at different concentrations (20, 40, 60, 80, 100 and 200) was carried out. Maceration extraction showed activity ranging from 45-80 % for fruit sample, 37-85 % for stem sample and 33-64% for leaf sample. The results are expressed in below table 7

Table 7 observed % Antioxidant activity of Maceration extraction of fruit, leaf and stem sample

Sr. No.	Concentration	% Antioxidant Activity		
		Fruit	Leaf	Stem
1	20	45	64.62	37.01
2	40	56.26	56.56	75
3	60	77.76	50.74	80.82
4	80	80.37	34.62	81.79
5	100	79.02	34.32	85.59
6	200	63.28	33.74	78.58

The absorbance was gradually increased with increasing concentrations at certain concentration and then decreases or remains same gradually over increasing concentration of maceration extracts. The antioxidant activity of different Maceration extracts as equivalent to DPPH was in the order of fruit > stem > leaf sample of M. nigra. Higher antioxidant activity is seen at 80, 20 and 100 µg/ml conc. whereas, low activity is seen at 20, 200 and 20 µg/ml conc. of fruit. Leaf and stem sample respectively.

The results reveal that antioxidant activity is higher in fruits and comparatively low in leaves of M. nigra. The graphic representation of comparison of % antioxidant activity of Maceration extract of fruit, leaf and stem sample was shown in below figure 6

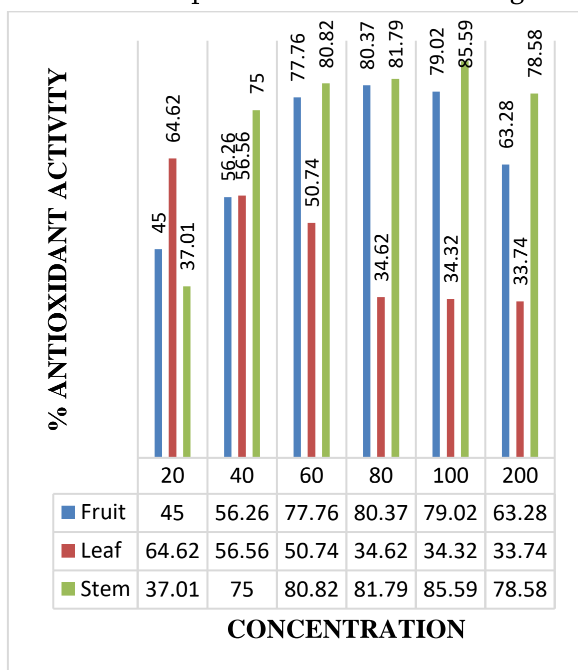

Figure 6 Comparison of % Antioxidant activity of Maceration extraction of fruit, leaf and stem sample

The antioxidant activity of Ultrasonication extracts from the fruit, leaf and stem samples of M. nigra at different concentrations (20, 40, 60, 80, 100 and 200) was carried out. Ultrasonication extraction showed activity ranging from14-26% for fruit sample,10-26% for stem sample and 8-21% for leaf sample. The results are expressed in below table 8

Table 8 observed % Antioxidant activity of Ultrasonication extraction of fruit, leaf and stem sample

Sr. No.	Concentration	% Antioxidant Activity		
		Fruit	Leaf	Stem
1	20	20.14	21.64	13.43
2	40	14.92	11.19	11.19
3	60	26.34	11.94	10.44
4	80	25.52	10.44	19.40
5	100	21.64	14.92	26.86
6	200	25.67	8.95	25.59

The absorbance was gradually increased with increasing concentrations at certain concentration and then decreases or remains same gradually over increasing concentration of ultrasonication extracts. The antioxidant activity of different Maceration extracts as equivalent to DPPH was in the order of fruit > stem > leaf sample of M. nigra. Higher antioxidant activity is seen at 80, 20 and 100 µg/ml conc. whereas, low activity is seen at 20, 200 and 20 µg/ml conc. of fruit. Leaf and stem sample respectively.

The results reveal that antioxidant activity is higher in fruits and comparatively low in leaves of M. nigra. And overall higher activity is seen in case of maceration extract and low activity is seen in ultrasonication extract. The results of free radical scavenging potentials of all extracts prepared from different techniques were found to be in the order of Maceration > Soxhlet > Ultrasonication extract.

Therefore, the antioxidant activity results for all crude extracts from frit sample are higher than that from stem and leaves sample. The graphic representation of comparison of % antioxidant activity of Maceration extract of fruit, leaf and stem sample was shown in below figure 7

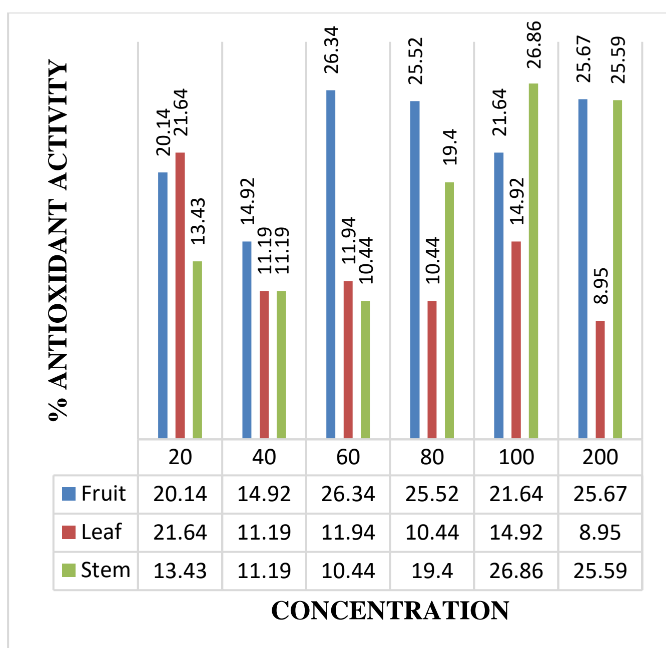

Figure 7 Comparison of % Antioxidant activity of Ultrasonication extraction of fruit, leaf and stem sample

Discussion

The phytochemical screening of Soxhlet, Maceration and Ultrasonication extracts from Fruit, stem and leaves samples of M. nigra used in this study revealed that the crude extracts contained alkaloids, flavonoids, glycosides, reducing sugars, tannins etc. as shown in table 6, 7 and 8. Therefore, the detected different bioactive compounds in different crude extracts from fruit, stem and leaves samples of M. nigra may be responsible for the antioxidant activity.

The antioxidant activity through free radical scavenging activity (DPPH) method of six different concentrations (20, 40, 60, 80, 100 and 200 μg/ml) extracts (prepared from Soxhlet, maceration and ultrasonication) from fruit, stem and leaves sample of M. nigra plant was determined and compared.

During the free radical reaction, DPPH (α, α - diphenyl-b-picrylhydrazyl) is converted into α, α - diphenyl-b-picrylhydrazine with colour change. The rate of colour change gradually decreases to indicate the scavenging potentials of the sample antioxidant.

The extracts of M. nigra contain flavonoid, tannins, phenolics and aromatic compounds. All these bioactive compounds were able to discolour DPPH solution by their hydrogen donating ability.

From the results it appears that the three different extracts from three different samples of fruit, stem and leaves of M. nigra possess hydrogen donating capabilities and it will act as an antioxidant. The results of free radical scavenging potentials of all sample extracts were found to be in the order of fruit > Stem > Leaves extract. And the results of free radical scavenging potentials of all extracts prepared from different techniques were found to be in the order of Maceration > Soxhlet > Ultrasonication extract.

The antioxidant activity for all fruit sample extracts was higher at low concentrations as compared to high concentrations. However no similar trends were obtained for all extracts from stem and leaf samples of M. nigra plant.

The activity difference obtained from different extract samples might probably be due to the extraction procedures, samples processing or drying. During the processing of samples some active volatile compounds may have been destroyed or evaporated from the samples. That may be why the antioxidant activity is differed for all different sample extracts.

IV. CONCLUSION

Phytochemical screening and comparative study of antioxidant activity of different parts of mulberry plant was done. The result obtained from this work prove that the three different extracts prepared from M. nigra have potential antioxidant property. The results reveal that sample show greater extent of activity in extract prepared from Maceration process as compared to Soxhlet and Ultrasonication. Whereas the sample shown greater extent of activity in fruit sample as compared to leaves and stem samples. It shows that the leaves, stems and

fruits can be considered as a source of natural antioxidant. Best technological condition and production factors are important for the productivity and bioavailability of herbal antioxidant used in food and biological systems. Hence the present work, demonstrate that the extract of Morus nigra have great antioxidant capacity determine by DPPH assay. Further research is needed towards isolation and identification of active principles present in the extracts which could be used for pharmaceutical use.

V. REFERENCES

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