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Primary Metabolite Profiling and Antioxidant Potential Assay from Selected Plant Parts of *Gmelina arborea*

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing various human diseases and also play an important role in healing as well as for curing human diseases. The present study involves different parts of medicinal plant Gmelina arborea for their primary metabolite profiling and antioxidant potential assay. The different plant parts were washed, air dried and then powdered. Primary constituents have chlorophyll, phenol, carbohydrates (Starch and Total soluble sugar), lipid and protein and antioxidant are Catalase, peroxidase, SOD and FRAP etc. The experimental plant has been used as traditional medicine in India for several medicinal purposes like anthelmintic, diuretic, antibacterial, antifungal, antioxidant and antidiabetic.

Keywords: *Gmelina arborea; Primary Metabolite; Antioxidants.*

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INTRODUCTION

Gmelina arborea *is a medicinal plant commonly known as "Gambhar" belongs to family Lamiaceae (Verbenaceae)*, is the most widely cultivated species of the genus Gmelina in the family *Lamiaceae,* a well-known medicinal plant in the India and Africa (1 - 4).

Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time-tested and comparatively safe for both human use and environment (5, 6). The medicinal plants are useful for healing as well as for curing human diseases because of the presence of phytochemical constituents (7). Phytochemicals occur naturally in the medicinal plants, leaves, vegetables and roots that have defense mechanism and provide protection from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, phenol, carotenoids, carbohydrates (Starch and Total soluble sugar), lipid and protein etc. are included in primary constituents and alkaloids, flavonoids, phytosterol, rotenoids and steroids etc. are included in secondary constituents (8). Free radical's oxidation has been implicated in atherosclerosis, cancers, neuron degenerative diseases and inflammatory bowel diseases (9, 10). The two most commonly used synthetic antioxidants are butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) having side effects of toxicity and DNA damage induction. Therefore, natural antioxidants from plants and algal extracts have attracted increasing interest due to their non-toxicity and biosafety (11). The fruit of the plant is used in treatment of scorpion sting, snake-bites (12) and diabetes (12, 13).

Synonyms of *Gmelina arborea*

In Indian, the synonyms of Gambhari in Vedas like Rohini, Kashmarya, Sriparni etc. The first time the name Rohini was mentioned in Atharva veda, where it is considered as Asthisansthapaka and Keetanunashaka. The name Kashmarya is given in Shatapatha Bramhan, where it is explained as disinfectant, but the properties of which do not correlate with the Gambhari (15). *Gmelina arborea* is also known by various synonyms, such as: *Gmelina arborea var. canescens* Haines, *Gmelina arborea* var. *glaucescens* C. B. Clarke, *Gmelina rheedei* Hook and *Gmelina sinuate.*

Vernacular name of *Gmelina arborea*

Gmelina arborea is locally called by different names in different languages in India (16) reported in **(Table 1)**.

Table 1: Vernacular name of *Gmelina arborea*	
Hindi	*Gamhar*
English	*Beechwood*
Sanskrit	*Gambhari*
Bengali	*Gumbar, gambar*
Kasmiri	*Mara, shivani*
Oriya	*Bhadraparni, kumar*
Punjabi	*Kumhar, gumhar*
Tamil	*Kumla, kumalamaram*
Telugu	*Gumartek, gummadi*
Marathi	*Shivan, siwan*
Malayalam	*Kumbil, pokki, kumbulu*
Gujarati	*Shewan, sivan*

Taxonomical Classification

The taxonomical hierarchy of *Gmelina arborea* (17) has been mentioned in **(Table 2)**

Table 2: Taxonomical Classification	
Kingdom	Plantae
Class	Angiosperms
Sub class	Eudicots
Super order	Asterids
Order	Lamiales
Family	Verbinaceae
Genus	*Gmelina*
Species	*Arborea*

Figure 1: *Gmelina arborea* Plant samples (Fresh leaves and Fruits)

Botanical Distribution

Gmelina arborea is a moderate sized unarmed deciduous tree 12–30m in height and 60–100 cm in diameter. Bark gray-yellow or light gray, thin, somewhat corking, smooth, becoming brown and rough, twigs stout, often slightly 4-angled. Leaves opposite, broadly ovate. Flowers are many, short-stalked, nodding and densely hairy. The drupe is fleshy, ovoid with 1 or 2 seeds (18).

Geographical Distribution

Gmelina arborea Roxb belonging to the family Verbenaceae fast growing deciduous tree found throughout India and also in Pakistan, Bangladesh, China, Japan, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand. It is a one of the herbs mentioned in all ancient scriptures of Ayurveda. It is notable to possess been utilized in ancient Indian drugs. It is a very important timber-yielding tree that grows naturally within the tropical and climatic zone regions of geographical region and has additionally been introduced as a plantation species outside these regions (19, 20).

Traditional Uses

It is used as a strengthening of the digestive system and as a tonic of the brain, it is also used in the form of tonic in many illnesses like heart disease and nervous system, apart from it, use of fever, snake bites, scorpions stings and piles, all of these statements have been mentioned in folklore states. Finally the whole parts of this tree have great importance role on medicinal level (21).

MATERIALS AND METHODS

Plant materials

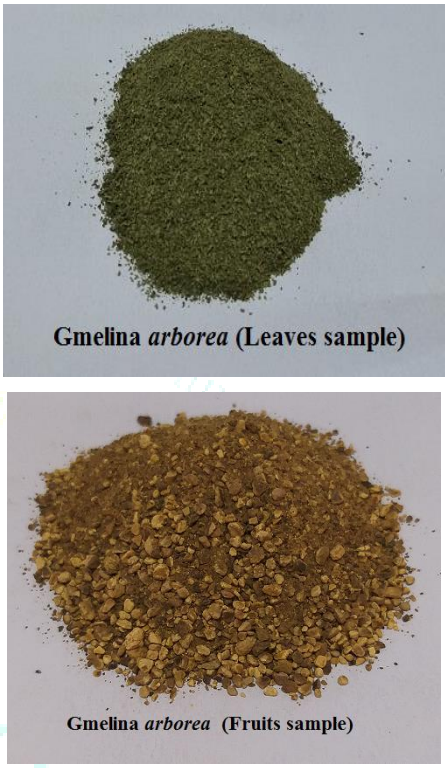

Figure 1: *Gmelina arborea* Plant samples (Dried powdered leaves and Fruits)

The present study involves different parts of medicinal plant *Gmelina arborea*. Experimental plants parts are collected locally from Botanical garden Department of forestry, District Palwal, state Haryana, India. The plant parts i.e. fresh leaf and fruits were used for the purpose of research work analysis. The leaf and fruits of selected medicinal plant parts were washed; air dried and then makes it powdered in (Fig. 1).

Chemicals use for experiments

Ethanol, distilled water, aqueous HCl, methanol, chloroform, concentrated sulphuric acid (H_2SO_4), Trichloroacetic acid (TCA), Na_2CO_3, $CuSO_4$, Sodium Potassium tartrat, perchloric acid ($HClO_4$), phenol, Folin & Ciocalteu's phenols, acetone and Sodium Carbonate.

Methods

Primary Metabolites

A primary metabolite is a kind of metabolite that is directly involved in normal growth, development, and reproduction of a plant. It's usually performs a physiological function in the organism (i.e. an intrinsic function). A primary matter is

often gift in several organisms or cells. Primary metabolites are like: Carbohydrates, proteins, phenol and lipids.

Carbohydrates

Sample preparation of plant materials for TSS and Starch estimations:

a) Total Soluble Sugars (TSS)

The dried plant materials leaf and fruits (50 gm each) were homogenized separately in a mortar and pestle with 20 ml of 80% ethanol and left overnight. Each of the samples was centrifuged at 1200 rpm for 15 minutes the supernatants were collected separately and concentrated on a water bath using method (22). Distilled water was another to create up the quantity up to 50 milliliter and processed additional for quantitative chemical analysis.

b) Starch

The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5.0 ml of 52% perchloric acid (23). Then, 6.5 ml of water was added to each sample and the mixture was shaken vigorously for 5 min.

Estimation procedure:

Total soluble sugar: - 80% ethanol use for extraction according protocol was followed using the method (22). 0.1 ml of sample was mixed with 5 ml of 80% ethanol reagent. Centrifuge at 10000 rpm for 20 min then supernatant collects in test tube. Add 5ml H_2SO_4 with 1ml 5% phenol then mix by vortex. Now kept sample at room temperature for 20 minutes. Absorbance was read at (wavlength) 490 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/gram dry weight sample.

Starch: - The protocol was followed using the method (23) for total soluble sugar. Take 5 ml of 80% ethanol in a test and mix with 0.1 ml plant sample, mix properly with the help of vertex and centrifuge at 10000 rpm for 20 minutes, collect pellet and mix with 1ml perchloric acid (HClO4) mix by vertex. Take 1 ml sample in test tube add 5ml H2SO4 and 1ml 5% phenol mixing by vortex keep 20 min room temp. The absorbance was scan at 490 nm against a chemical agent blank. The analysis was performed in triplicates and the results were expressed as mg/g dry weight.

Protein Estimation: -

10% TCA use for protein extraction according here methodology of (24) was followed. Take 0.1 ml of sample mixed it with 3ml 10% TCA, centrifuge at 15000 rpm for 10 minutes, now take pellet add 10 ml 5% TCA mix it by vortex. Now take in a test tube and incubate at 80 °C for 30 minutes, after incubation cool it and take 1 ml sample from it and add 5 ml alkaline solution with 1 ml Folin & Ciocalteu's reagent and incubated again for 10 minutes at 37 °C or room temperature. Absorbance was read at 750 nm (wavlength) against 10% TCA reagent blank. The analysis was performed in triplicates and the results were expressed mg/g dry weight sample.

Lipid Estimation: -

Distilled water is used for lipid extraction according extraction methodology (25) will be followed. Take 0.3 gm sample with 10 ml distilled water and crush it with the help of mortar and pestle. Add 20 ml chloroform ($CHCl_3$) with 10 ml methanol (CH_3OH) for 20 min kept on room temperature will filter it after 20 min. Now add 20 ml $CHCl_3$ with 2ml distilled water then proper mixing. Take in separating flask and collect lower layer. Dry it here blank weight less in dry weight take result. The analysis was performed in triplicates and the results were expressed mg/g dry weight sample.

Phenol Estimation: -

80% ethanol is used for extraction total phenol content in each sample was estimated by spectrophotometer method (26). Take 0.2 gm sample with 4 ml 80% ethanol crush it with the help of mortar and pestle. Centrifuge at 10000 rpm for 10 minutes and collect supernatant and take 1 ml of sample added 1 ml of Folin & Ciocalteau reagent and incubated at room temperature for 3 minutes. After three minutes 2 ml of 20% sodium carbonate (Na2CO3) was added, mixed well and incubated the tubes in boiling water bath for 1minute. Cooled rapidly and read absorbance at 750 nm (wavlength) against reagent blank. The analysis was performed in triplicates and therefore the results were expressed as mg/g sample.

Antioxidant activity

Antioxidants are compounds that inhibit oxidation. Oxidation could be a chemical action that may turn out free radicals, thereby resulting in chain reactions that will harm the cells of organisms. Antioxidants like thiols or antioxidant (vitamin C) terminate these chain reactions. In future the plant extract are significant sources of natural antioxidant, which may be helpful in preventing the progress of various oxidative stresses and as a possible food supplement or in pharmaceutical industry. Antioxidants are like: DPPH, FRAP, Catalase, LPO and peroxidase etc.

DPPH (2, 2-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity was measured using the stable free radical DPPH which is one of the main tests used to explore the use of herbal extracts as antioxidants, the experimental protocol followed (27). DPPH is solubilized in methanol to have a solution of 0.3 mM. 1 ml of methanol and 1 ml of the extract (at different concentrations 1 mg / ml in methanol) are introduced into tubes and 2 ml of the methanol solution are added to the DPPH. After stirring by a vortex, the tubes are placed in the dark at room temperature for 30 min. The reading is performed by measuring the absorbance at 517 nm.

FRAP (Ferric reducing antioxidant power)

The FRAP assay was used to estimate the reducing capacity of plant extracts, according to the method (28). The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM FeCl3.6H2O and 25 mL of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37ºC temperature. 900 µl FRAP chemical agent was mixed with 90 µl water and 30 µl of the extract. The reaction mixture was incubated at 37ºC temperature for 30 minutes and the absorbance was measured at 593 nm.

Catalase

Catalase activity in our laboratory is measured by a spectrophotometric procedure measuring peroxide removal. It is a direct assay with pseudo-first order kinetics and is measured by the method (29). The enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H2O2, 0.4 ml H2O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the management, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and therefore the absorbance was scan at 610 nm.

Catalase activity was expressed in terms of µmoles of H2O2 consumed/min/mg macromolecule.

LPO (Lipid peroxidase)

Homogenize 0.1 gram of leaf tissue by adding 0.5 ml 0.1 % (w/v) TCA. Centrifuge the homogenate for 10 min (15000 rpm at 4.0oC). Collect supernatant and mix 0.5 ml of supernatant with 1.5 ml 0.5% TBA diluted in 20 % TCA. Incubate in water bath at 95o C for 25 min. End reaction by incubating on ice. In case the solution is not clear, centrifuge for a further 5 min (15000 rpm at 4.0 ^{0}C). Measure the absorbance at 532 and 600 nm (30) (Health & Packer, 1968).

Peroxidase

The peroxidase assay was carried out by the method (31). The reaction mixture consisted of 3ml of buffered pyrogallol (0.3ml of 0.05 M pyrogallol in 2.4ml of 0.1 M phosphate buffer (pH 7.0). add 0.5 ml of 1% H2O2 and add 0.1 ml plant extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The oxidase activity was calculated victimisation associate extinction constant of alter phenol (4.5litres/mol).

Superoxide Dismutase

Superoxide dismutase (SOD) activity was determined by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme method (32). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 µM NBT, 10 µM riboflavin and 100 µL of crude extract during a final volume of 3.0 mL. A control reaction was performed without crude extract. The SOD reaction was dispensed by exposing the reaction mixture to white light-weight for 15 min at room37 ^{0}C temperature. After 15 min incubation, absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was outlined because the quantity of protein inflicting 500th inhibition of chemical science reduction of NBT

RESULT AND DISCUSSION

Medicinal plants are imported role to health of individual and communities. The medicinal activities of experimental plant parts are some chemical substances that produce a definite physiological action to the human body. Phytochemicals analysis is of paramount importance in identifying a new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated. In the present study estimate primary metabolites was qualitatively, quantitatively and antioxidant assay analyzed using *Gmelina arborea* leaves and fruits.

Primary metabolite

Total level of primary metabolites (mg/gram dry weight) in various plant parts (table 3)

Primary Metabolites extracts from ***Gmelina arborea*** leaf and fruits **(mg/gram dry weight)**					
	Total Soluble Sugar	**Starch**	**Protein**	**Phenols**	**Lipids**
Leaves extract	**1.42**	**1.48**	**1.76**	**2.85**	**3.33**
Fruits extract	**1.38**	**1.58**	**1.78**	**110**	**23.33**

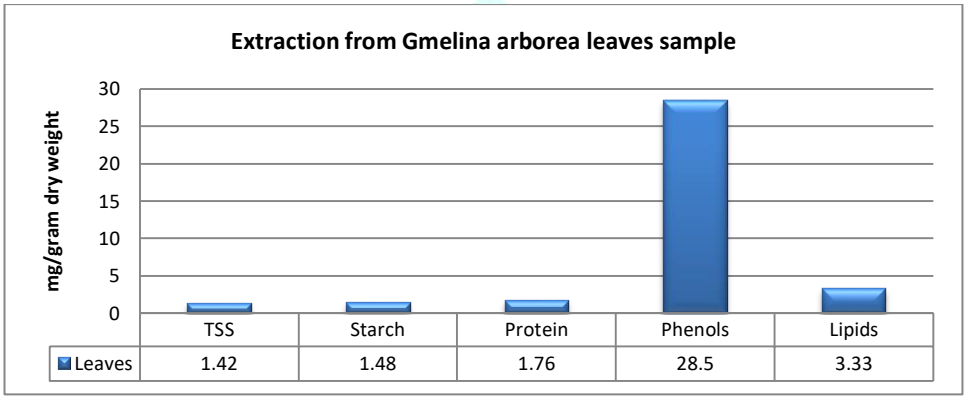

Figure 3: Graphical presentation of primary metabolites leaf extract

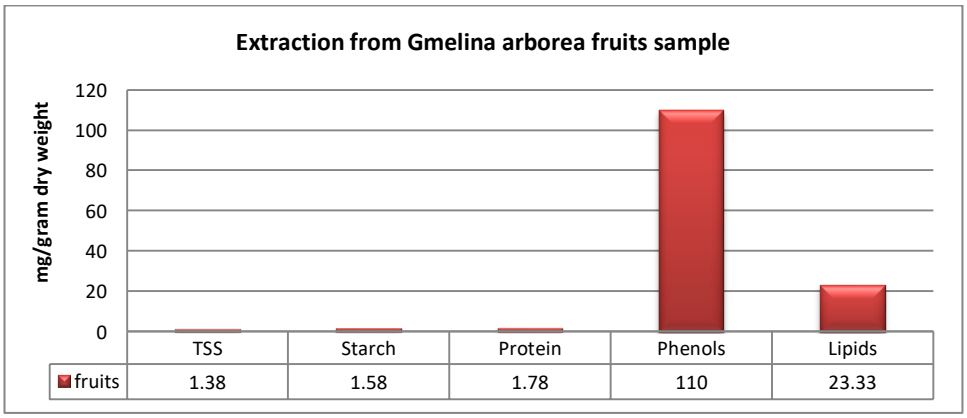

Figure 4: Graphical presentation of primary metabolites leaf extract

Antioxidants

Total level of Antioxidant assay (µM / L / g fresh weight) in Leaf and Fruits extract shown in following table.

Plant Extract	POD	SOD	CAT	FRAP	LPO	DPPH
Leaves Extracts	0.67	1.04	0.92	0.92	7.63	35.82
Fruits Extracts	1.52	1.493	1.24	0.51	8.37	27.4

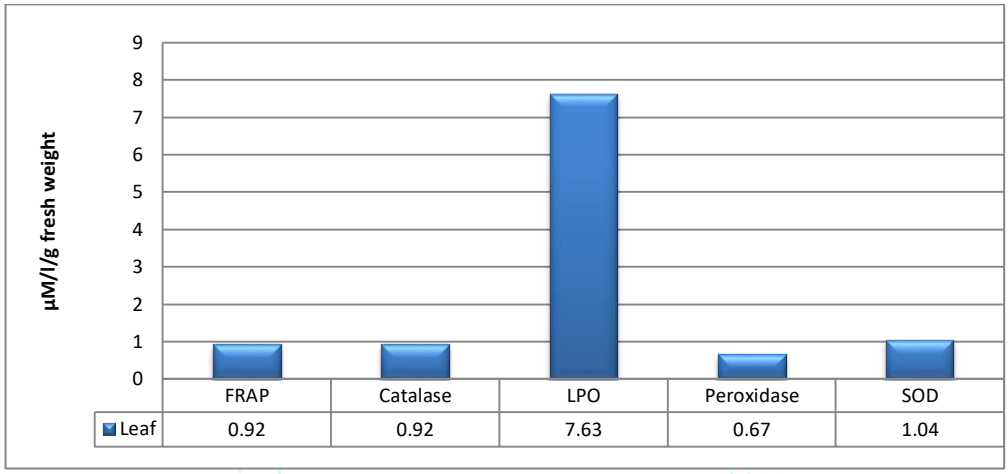

Figure 5: Total level of Antioxidant assay (µM / L / g fresh weight) in Leaf extract

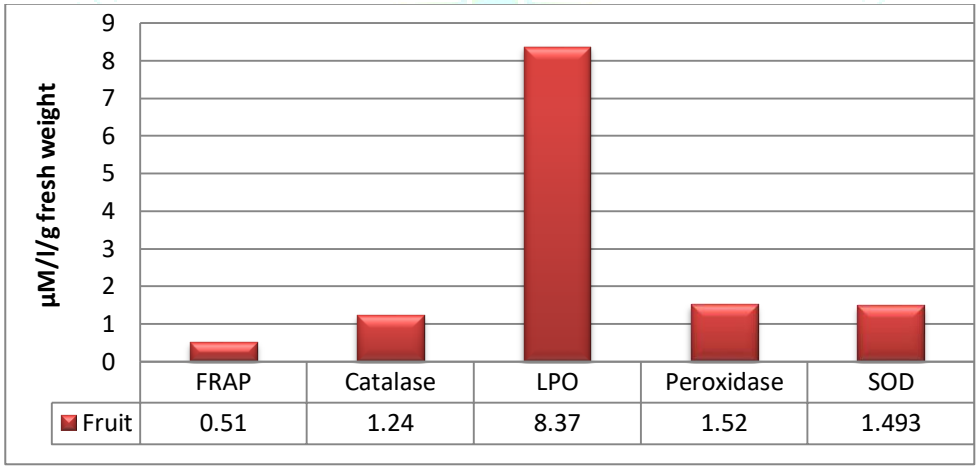

Figure 6: Total level of Antioxidant assay (µM / L / g fresh weight) in Fruit extract

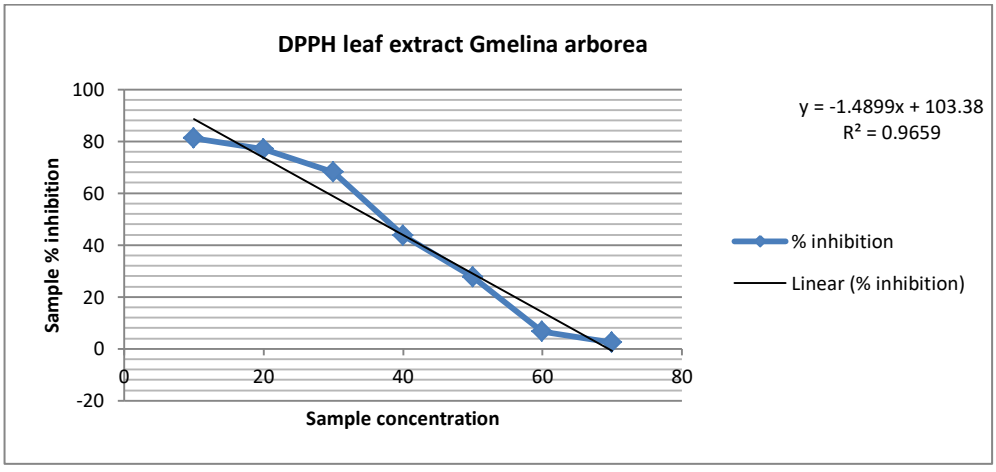

Figure 7: Graphical presentation to DPPH Leaf extraction result of *Gmelina arborea*

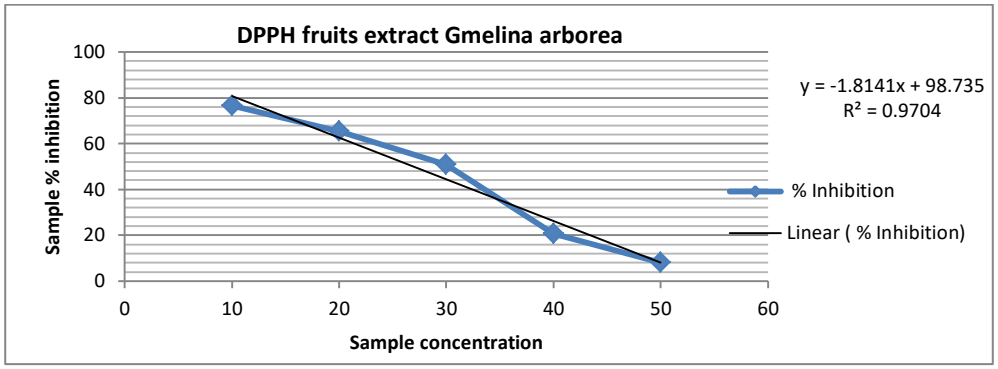

Figure 8: Graphical presentation to DPPH Fruits extraction result of *Gmelina arborea*

CONCLUSION

The present study indicates the importance of selective experimental plant *Gmelina arborea* (*Gambhari*), as one of the classical medicinal plants. *Gmelina arborea* is a fast growing and multipurpose medicinal plant, we determined different Primary metabolites (Carbohydrates, proteins, phenols and lipids) and antioxidant enzyme activities (DPPH, FRAP, CAT, POD, SOD and LPO) in experimental plant (*G. arborea*). Our results showed that (Fig. 1-8) has a good phytochemicals and antioxidant potential. Based on the results, we can conclude that the plant is a promising source of energy supplements or natural nutrients, antioxidants and might be used in the treatment of human diseases.

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