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Rohit Kumar Bargah
Assistant Professor & Head, Department of Chemistry, Government SPM College, Sitapur, Surguja, Chhattisgarh, India

Pradeep Singh Painkra
Research Scholar, Department of Chemistry, Government SPM College, Sitapur, Surguja, Chhattisgarh, India

Prabha Wati Painkra
Research Scholar, Department of Chemistry, Government SPM College, Sitapur, Surguja, Chhattisgarh, India

Ankit Kumar Soni
Research Scholar, Department of Chemistry, Government SPM College, Sitapur, Surguja, Chhattisgarh, India

Sanjeeta Tirkey
Research Scholar, Department of Chemistry, Government SPM College, Sitapur, Surguja, Chhattisgarh, India

Harawati Painkra
Research Scholar, Department of Chemistry, Government SPM College, Sitapur, Surguja, Chhattisgarh, India

Punita Bhagat
Research Scholar, Department of Chemistry, Government SPM College, Sitapur, Surguja, Chhattisgarh, India

Corresponding Author:
Rohit Kumar Bargah
Assistant Professor & Head, Department of Chemistry, Government SPM College, Sitapur, Surguja, Chhattisgarh, India

Qualitative and quantitative phytochemical analysis and *in vitro* antioxidant activity of flowers extract from *Gmelina arborea* Roxb. (Verbenaceae)

Rohit Kumar Bargah, Pradeep Singh Painkra, Prabha Wati Painkra, Ankit Kumar Soni, Sanjeeta Tirkey, Harawati Painkra and Punita Bhagat

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Abstract
Plants are considered to be a promising source of medicine in the traditional health care system. Now days, there is a revival of interest with herbal-based medicine due to the increasing realization of the health hazards associated with the indiscriminate use of modern medicine. The aims of present investigation are to carried out phytochemical screening and quantify phytoconstituents of *Gmelina arborea* (Verbenaceae) flowers extracts. The quantification of total phenolic and flavonoids were carried out using Folin-Ciocalteu method and aluminium chloride method respectively in G. arborea flower extracts. Antioxidant activities of the selected samples were tested using 1-diphenyl-2-picrylhydrazyl (DPPH) Assay. The result revealed the presence of flavonoids, saponins, Tannins, Triterpenoids, Reducing Sugar, Phenolic compound, steroids in methanol and aqueous extract but Anthraquinones Glycoside were absent in methanol and aqueous extract. Total phenol content was expressed in mg of Gallic Acid Equivalent (GAE) per g of dry weight. In results it was found that methanol extract shows highest phenol content 365.94±0.26 mg/g in flowers. The content of flavonoids was expressed in mg of Quercetin Equivalent (QE) per g of dry weight. It was evaluated that total flavonoid content found highest in flowers was 335.24±0.16 mg/g in methanol extract. The Results revealed that methanol extract showed the highest amount of phenolic and flavonoids content was found in methanolic extract of flowers. IC_{50} for standard ascorbic acid was found to be 51.36 µg/ml and for methanol and aqueous extract flower was found to be 88.20 µg/ml and 130.01 µg/ml respectively. The DPPH radical scavenging activity of *Gmelina arborea* was evaluated and compared with ascorbic acid. The presence inhibition of flower extract was calculated at various concentrations (50, 100, 150, 200, 250 etc.) as well as standard ascorbic acid. The highest scavenging activity of methanolic and aqueous extract were 95.11±0.64% and 82.43±0.63% at concentrations of 250 µg/ml. The present research could be useful for isolation and identification of specific phytoconstituents responsible for particular pharmacological activity. It was concluded that *Gmelina arborea* extracts possess a powerful bioactive compound and antioxidant activities.

Keywords: *Gmelina arborea*, antioxidant activity, DPPH total phenolic content, total flavonoid content phytochemical screening

1. Introduction
The plant kingdom is a treasure house of potential drugs. Currently, there has been a rising awareness about the importance of medicinal plants. The plant drugs are easily obtainable, not as much of expensive, safe and original. The plants have been used for medicinal purposes during from thousands of years, and the clearest option today is to explore for therapeutically effective new drugs. According to the World Health Organization (WHO), medicinal plants are the source to obtain the variety of drugs. About 80% of folks within developed countries are using traditional medicines and this has derived compounds from medicinal plants [1].

Nature has provided a complete storehouse of remedies to cure all ailments of mankind. About 80% of the world population depending on herbal based alternative system of medicine (Ayurveda, Unani medicine and Chinese traditional medicine) [2-3]. Herbal drugs have played a vital role in curing diseases throughout the history of mankind.

Despite the major advances in modern medicine, the development of new drugs from natural products is still considered important [4-5]. The evaluation of various plant products according to their traditional uses and medicinal value based on their therapeutic efficacy leads to the discovery of newer and recent drugs for treating various ailments. This fact forms the basis for the development of new drugs from various plant sources [6]. In India, huge amount of medicines had been used from plants or their extracts. Still many more plants warrant such expeditions G. arborea is one such tree species need to be studied for the therapeutically effective drug [7-8].

Gmelina arborea (Roxb.), belonging to the family Verbenaceae, commonly known as 'Gamhar'. Gmelina is one of the important genera of the family, consisting of about 33 species. It is deciduous tree with smooth whitish grey bark. Fruits are drupe, 1.8-2.5cm long, obovoid, bear the enlarged calyx; yellow when ripe. Endocarp is bony and 2-celled. Seeds are reddish in colour, lenticular and exalbuminous [9]. Bark, wood, leaf, root and fruits are used in treatment of various ailments in folk medicine [10-11].

The tree is also an important medicinal plant in the Indian Systems of Medicine. The whole plant is used in medicine. It is astringent, bitter, digestive, cardio tonic, diuretic, laxative and pulmonary and nervine tonic. It improves digestion, memory, helps overcome giddiness and is useful in burning sensation, fever, thirst, emaciation, heart diseases, nervous disorders and piles [12-13]. The roots are acrid, bitter-sweet in taste, stomachic, tonic, laxative, galactagogue and antihelmintic.

Fig 1: Flowering plant of *Gmelina arborea*

The flowers are sweet, refrigerant, bitter, astringent and acrid, and are used in treating leprosy and skin diseases. The fruits are acrid, sour, sweet, refrigerant, bitter, astringent, aphrodisiac, trichogenous, alterant and tonic. Fruits are edible and also used for promoting hair growth and in treating anaemia, leprosy, ulcers, constipation, leucorrhoea and colitis. The leaves are a good fodder also. The major bioactive compounds extracted from different parts of *G. arborea* are arboreal, verbascoside, tyrosol, iridoids, phenylpropanoid glycoside, premnazole, martynoside, iridoid glycosides, balanophonin, gmelinol, isoarboreol apigenin, umbelliferone etc. [10].

2. Methods and Materials

2.1 Collection of plant material

The fresh flowers parts were collected from the Mainpat forest region of Surguja, District of Chhattisgarh India in the month of February-March 2024 and the plant material was authenticated by fthe taxonomist Prof. Rijwan Ulla, Department of Botany, Rajeev Gandhi Govt. Autonomous Post Graduate College Ambikapur, Surguja, Chhattisgarh, India.

2.2 Preparation of extract

250 gm. of powdered of *Gmelina arborea* were packed in soxhlets apparatus separately and extracted with solvent methanol and aqueous. The extracts were filtered while hot and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The methanolic and aqueous extracts were stored in refrigerator for further experimental work [14].

2.3 Qualitative Chemical Test

The Flowers extracts obtained by solvent extraction from G. arborea subjected for qualitative chemical tests for phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins, and Phytosterols. Preliminary phytochemical screening was performed by using standard method [15-21].

Tests for Alkaloids

- **Dragendorff's test:** 1 ml of extract. + 1 ml KBr. An orange-red ppt. (alkaloids present).
- **Mayer's test:** 1 ml of extract + 1 ml of KHgI. Whitish yellow or cream-colored ppt. (alkaloids present).

Test for Glycosides

- **Legal's test:** (1 ml Ext. + Pyridine + Na_2 [Fe (CN) $_5$NO]). No colour (Glycoside absent).
- **Baljet's test:** (1ml ext. + 1 ml $C_6H_2KN_3O_7$) yellow to orange colour (glycoside present).

Tests for Carbohydrate

- **Benedict's test:** (5ml Benedict's reagent. + 1ml extract) Boil 2 min. and cool. (Red ppt., sugars present).
- **Molisch's test:** Extracts in ethanol separately + drops of 20% w/v solution of α naphthol in ethanol (90%). Shake well + add from side of test tube 1 ml of Conc. H_2SO_4 was Reddish violet ring between junction of the layers (carbohydrates present).

Test for Steroids

- **Salkowski test:** (Extract + $CHCl_3$ + equal volume of conc. H_2SO_4) was added. Bluish red to cherry in $CHCl_3$ and green fluorescence in the acid (steroidal present).
- **Liebermann-Burchard test**: (Extract + 1 ml of acetic anhydride and dissolved) by warming. The contents were cooled and a few drops of conc. H_2SO_4 were from the sides of the test tube. (Blue colour) sterols present.

Test for Proteins

Biuret test: 1ml 40% NaOH + 2 drops 1% $CuSO_4$ solution. Till a blue color appear + 1ml extract. Pinkish / purple violet color (protein present).

Test for Saponins

- Extracts boiled with 1 ml of distilled water and shaken. Foam formed (saponins present).
- Extract + 2 ml of Distilled Water + sodium carbonate and shake. The Foam formed (saponins present).

Test for Tannins

- Extract + lead acetate solution. White precipitates (Tannins present).

Tests for Flavonoids

Shinoda test: Test solution + magnesium turnings Conc. HCl drops pink scarlet.

Test for phenolic compounds: Small amount of extract was mixed with few drops of freshly prepared 5% $FeCl_3$ solution; deep blue-black colour indicated the presence of phenolic compounds. Small amount of extracts mixed with lead acetate solution, formation of white ppt. indicated the presence of phenolic compounds.

2.4 Quantification of phytoconstituents

Preliminary phytochemical screening of *Gmelina arborea* flowers for phenolics and flavonoids were done by following methods**.**

Total Phenolics Content (TPC)

Total Phenolic content was determined in methanol extract, ethyl acetate extract, water extracts of fruits G. arborea by Singleton and Rossi (1965). Total phenolic was determined with Folin-Ciocalteu reagent using Gallic acid as a standard phenolic compound. The calibration curve of Gallic acid was taken using methanol. The 1.0 ml extract solution containing 5mg extract was diluted with 10 ml of distilled water. To this, 1.5 ml of Folin-Ciocalteu reagent was added. The above mixture was kept for 5 min. and then 4 ml of 20% sodium carbonate solution was added and made the volume up to 25 ml with the distilled water. This mixture was kept for 30 min and the absorbance of the blue color developed was measured at 765 nm, using Shimadzu 1800 spectrophotometer. The percentage of total phenolics was expressed as % Gallic acid [22-23].

Total Flavonoid Content (TFC)

Flavonoids with various biological activities are considered as the key compounds in the plants. Flavonoids content was determined in methanol extract, ethyl acetate extract, water extract of fruits on G. arborea using aluminium chloride method. Quercetin was used as a standard phenolic compound. The solution of plant extract and quercetin were prepared using methanol. The 1.0 ml extract solution containing 5mg extract was mixed with 1.5ml 95% methanol, 0.1ml 10% aluminium chloride, and 0.1ml 1M potassium acetate and 2.8 ml distilled water. After incubation at room temperature for 30 min, the absorbance of reaction mixture was measured at 415 nm using a colorimeter. Blank solution was prepared substituting the amount of 10% aluminium chloride by the same amount of distilled water. Calibration curve was prepared using concentration of quercetin verses absorbance. The percentage of total flavonoids was expressed as the percentage of quercetin [24-25].

2.5 DPPH (1, 1-diphenyl-2 picrylhydrazyl) radical scavenging activity

1, 1-diphenyl 1-2 picryl hydrazyl (DPPH) radical scavenging activity was measured according to the standard method [23]. This assay was used in many studies for testing antioxidant activity. 2, 2-diphenyl-1-picryl-hydrazil stable radical (DPPH) evidently offers a convenient and accurate method for titrating the oxidizable groups of natural and synthetic antioxidants. This assay was based on the reduction of a methanolic solution of the collared free radical DPPH by free radical scavenger. The degradation of DPPH was evaluated by comparison with a control sample without hydrogen-donating compounds. The decrease in absorbance of DPPH at its absorbance maximum of 517 nm was proportional to the concentration of free radical scavenger added to DPPH reagent solution. Lower absorbance of reaction mixture indicated higher antioxidant activity [26].

In this experiment methanolic solution of DPPH (100 mM, 2.95 ml), 0.05 ml of each extracts dissolved in methanol was added at different concentrations (50-250 µg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into a percentage of antioxidant activity (% AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation [27].

$$\text{DPPH scavenging effect} = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} \times 100$$

Where, A Control and A Sample standard for absorbance of the control and absorbance of tested extract solution respectively.

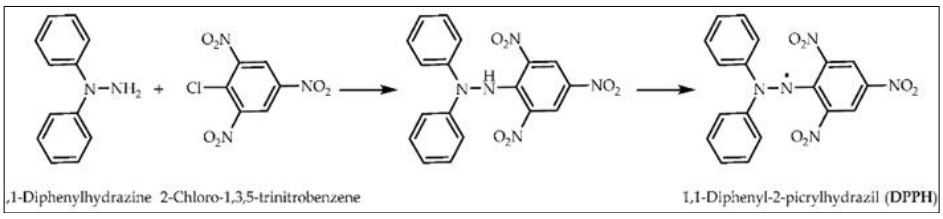

Fig 1: The synthesis route of 1, 1-diphenyl-2-picrylhydrazyl radicals (DPPH)

The antioxidant capacity of test samples was expressed as IC_{50} the concentration necessary for 50% reduction of DPPH. IC_{50} values denote the concentration of sample required to scavenge 50% of DPPH free radicals. IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. The measurements were triplicates and their scavenging effect was calculated based on the percentage of DPPH scavenged [28-31].

3. Results and Discussion

3.1 Qualitative chemical test

The phytochemical analysis is commercially important for pharmaceutical industries to the production of new drugs to

cure a variety of diseases. The secondary metabolites are significantly contributing to the biological activities and the plant materials contain numerous types of antioxidants with varied activities. The present study revealed that the presence of carbohydrates, phenolic compound, alkaloids, flavonoids, tannins, saponins, terpenoid and sterols in the flower of the *Gmelina arborea*.

Table 1: Phytochemical screening of *Gmelina arborea* flower methanolic and aqueous extract

S. N	Phytochemicals	Methanolic Extract	Aqueous Extract
1	Flavonoid	+	+
2	Saponins	+	+
3	Tannins	+	+
4	Steroidal Glycoside	+	+
5	Triterpenoids	+	+
6	Anthraquinones Glycoside	-	-
7	Reducing Sugar	+	+
8	Alkaloid	+	+
9	Steroids	+	+
10	Phenolic compound	+	+

(+): Presence, (-): Absent

3.2 Quantitative Chemical Test

The results given in table 3.2 show that the total phenol content and total flavonoid of *Gmelina arborea* flowers are methanolic 365.94± 0.26, 335.24± 0.16 and aqueous extract 295.20± 0.44, 255.75± 0.20 respectively.

Table 2: Result of quantification studies *Gmelina arborea* flower

S. N.	Extract	Test Parameter	Results (mg/g), (±SEM)
1.	Methanol	Total phenolic	365.94±0.26
		Total Flavonoids	335.24±0.16
2.	Aqueous	Total phenolic	295.20±0.44
		Total Flavonoids	255.75±0.20

3.3 DPPH scavenging activity of *Gmelina arborea*

DPPH is a stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichiometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. As shown in table *Gmelina arborea* of methanol extracts strongly scavenged DPPH radical with the IC_{50} being 88.20 µg/ml, respectively (Figure 3.4). The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 91.53±0.81.

DPPH scavenging activity of *Gmelina arborea* flower extracts against DPPH radical were determined and the results are shown in table (3.3, 3.4, 3.5). DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the *in vitro* antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. IC_{50} for standard ascorbic acid was found to be 51.36 µg/ml and for methanol and aqueous extract flower was found to be 88.20µg/ml and 130.01 µg/ml, respectively. In order to study the effects of these compounds on biological system more studies are needed as these compounds might be responsible for use of this plant in different diseases [32]. The DPPH radical scavenging activity of *Gmelina arborea* was evaluated and compared with ascorbic acid.

The presence inhibition of flowers extract was calculated at various concentration (50, 100, 150, 200, 250 etc.) as well as standard ascorbic acid. The highest scavenging activity of methanolic and aqueous extract were 95.11±0.64% and 82.43±0.63% at concentration of 250 µg/ml. Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups [33]. The polyphenolic compounds may contribute directly to the antioxidative action [34].

Table 3: Free radical scavenging capacity of Ascorbic acid

Concentration (µg/ml)	DPPH Scavenging %
	Methanol Extract
50	51.2±0.36
100	86.7±0.27
150	129.8±0.98
200	167.1±0.24
250	212.3±0.76
IC_{50}	51.36

Values are mean ± SEM of three determinations

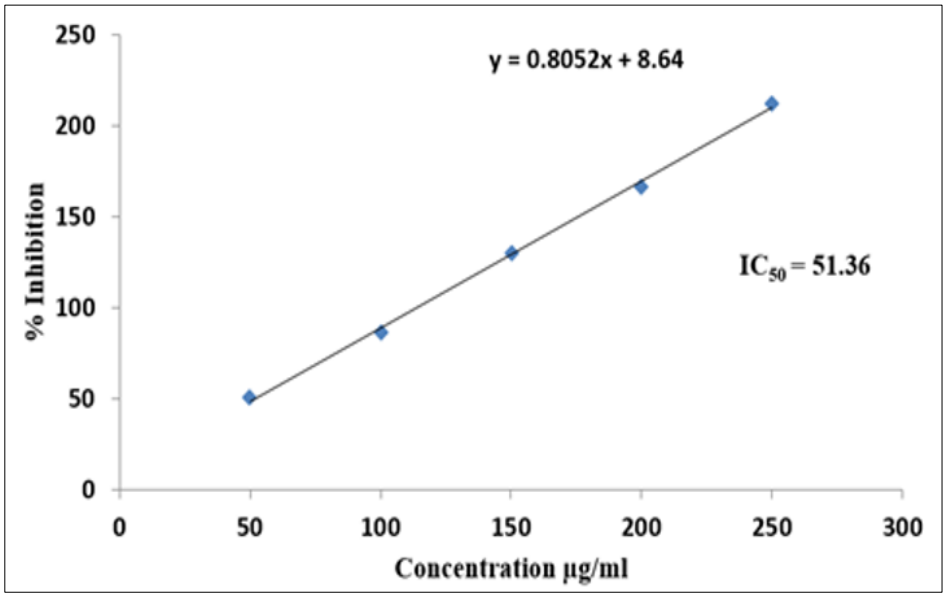

Fig 2: Free radical scavenging capacity of Ascorbic acid

Table 4: Free radical scavenging capacity of methanol extract of *Gmelina arborea* flower

Concentration (µg/ml)	DPPH Scavenging %	
	Methanol Extract	Ascorbic Acid
50	38.17±1.05	91.53±0.81
100	53.62±0.62	-
150	68.12±0.84	-
200	84.36±0.42	-
250	95.11±0.64	-
IC_{50}	88.20	-

Values are mean ± SEM of three determinations

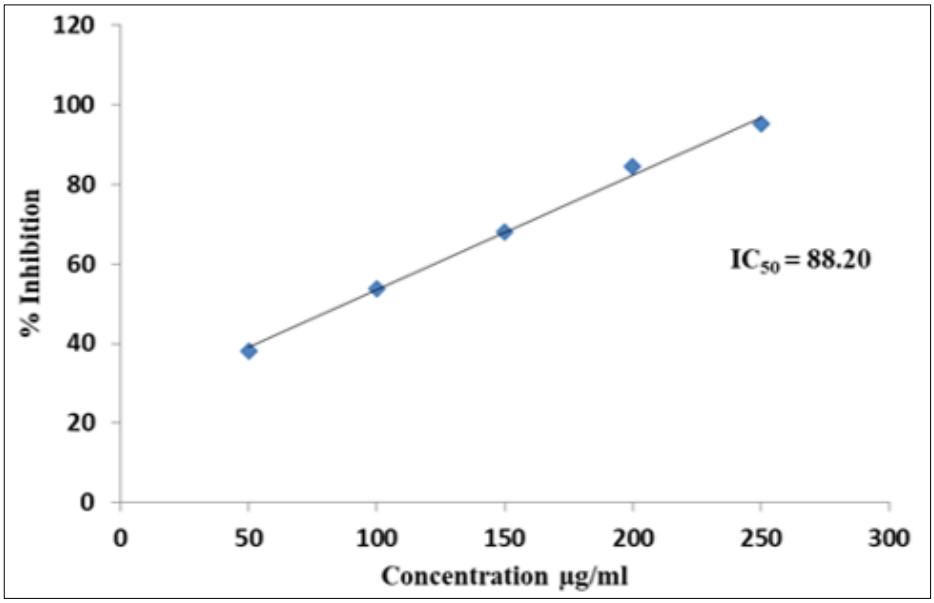

Fig 3: % DPPH scavenging of methanol extract

Table 5: Free radical scavenging capacity of aqueous extract of *Gmelina arborea* flower

Concentration (µg/ml)	DPPH Scavenging %	
	Aqueous Extract	Ascorbic Acid
50	27.14±0.38	91.53±0.81
100	41.85±0.72	-
150	55.24±0.49	-
200	71.30±0.35	-
250	82.43±0.63	-
IC_{50}	130.01	-

Values are mean ± SEM of three determinations

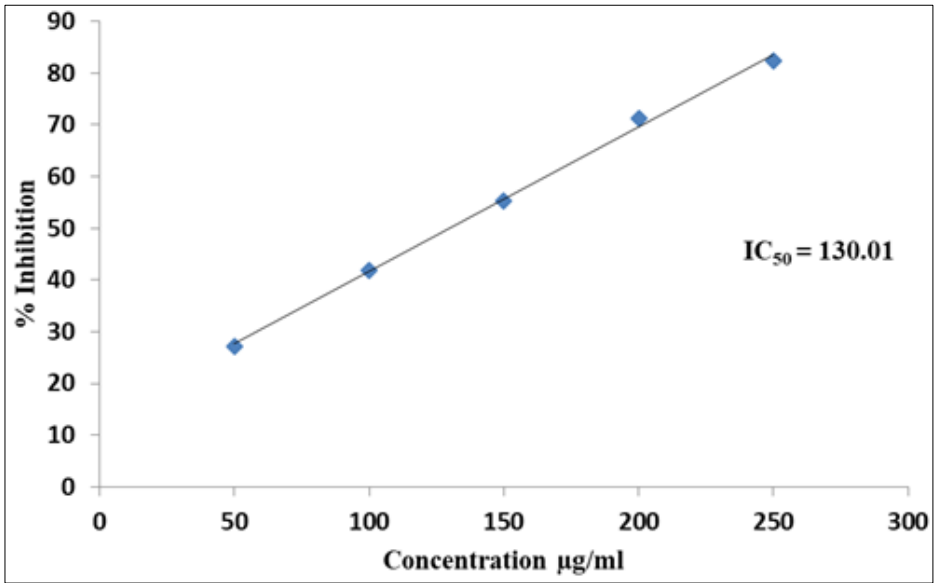

Fig 4: % DPPH scavenging of Aqueous extract

The DPPH antioxidant assay is based on the principle that 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, is able to decolourise in the presence of free radical scavengers (Antioxidants). The odd electron in the DPPH radical is responsible for the absorbance at 517 nm, and also for visible deep purple colour. When DPPH accepts an electron donated by a free radical scavenger, the DPPH is decolorized, and the extent of decolourisation can be quantitatively measured from the changes in absorbance [35-36]. It was found that the radical-scavenging activities of both extracts increased with increase in concentrations of extracts. It showed significant activity. The antioxidant activity of *Gmelina arborea* is mainly due to the presence of phenolic compounds [37-38].

4. Conclusion

Gmelina arborea is one of the trees that are found to have a number of medicinal and therapeutic properties. The present study has focused on a comparative view of the phytochemical concentration and antioxidant properties of flowers parts of the plant. Flowers were the best sources of phenolics and flavonoids. Further analysis is recommended to prove antioxidant properties of the plants under study, for better understanding and to implement them as potent antioxidant drugs. The results are very much encouraging but advanced work is necessary before being put into practice.

5. Conflict of interest statement

We declare that we have no conflict of interest.

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