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Research Article

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Evaluation of Physicochemical, Phytochemical Profile, In-vitro Anti-oxidant, Anti-inflammatory activity and FTIR Spectroscopic Analysis of *Acacia auriculiformis* Benth. flowers Extract

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Abstract

Medicinal plant formed the basis and foundation stone of diseases from the very beginning of human civilization. Medicinal component from plants play many important roles in traditional medicine. People in all around the worlds have long been applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory.

The objective of the present study was to evaluate Physicochemical, phytochemical, antioxidant, anti-inflammatory activities and FTIR Spectroscopic of methanolic and aqueous flower extract of *Acacia auriculiformis*. The solvents methanol and aqueous are used for extraction of *Acacia auriculiformis* plant parts. Phytochemical screening of plant parts was carried out in both the solvents. Determination of total phenol content was carried out using Folin - Ciocalteau method and total flavonoid content using Aluminium chloride spectrophotometric method. Antioxidant activity of methanol extract of plant samples were evaluated with DPPH and standard method. Anti - inflammatory activity of the flower parts the plant were analysed by *an In-vitro* protein denaturation inhibition assays. The FT-IR is a very useful technique for identifying the functional groups present in the mixture.

The result revealed the presence of flavonoids, alkaloids, Triterpenoids, Phenolic compound, Steroidal glycosides, saponins, tannins, Reducing Sugar, in methanol and aqueous extract. Total phenol content and total flavonoid of *Acacia auriculiformis* flowers are methanol 363.94± 0.17, 325.25 ± 0.14 and aqueous extract extract 275.20± 0.34, 253.72± 0.25 respectively. In results it was found that methanol extract shows highest phenol content and total flavonoids. *Acacia auriculiformis* flower extract of methanol and aqueous extracts strongly scavenged DPPH radical with the IC_{50} being 87.25 µg/ml and 125.01 µg/ml respectively. The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 90.53±0.82. The protein denaturation assay is used to evaluate anti-inflammatory activity. The results show a concentration-dependent increase in inhibition for all three samples. The methanolic extract exhibited higher activity than the aqueous extract at every concentration. At 100 g/L, the methanolic extract showed `86.70 ± 0.9%` inhibition, while the aqueous extract showed `65.45 ± 0.3%`. Sodium Diclofenac, a standard NSAID drug used as the positive control, demonstrated the highest activity, reaching `120.40 ± 0.6%` at 100 g/L. FT-IR analysis confirmed the presence of various functional groups.

Acacia auriculiformis is a valuable medicinal and industrial plant noted for its environmental adaptation and therapeutic value. The *Acacia auriculiformis* includes a wide range of phytochemicals, including flavonoids, tannins, saponins, phenolic acids, terpenoids, and sterols, all of which are linked to various biological functions. With further evaluation towards other biological and pharmacological activity of the tested plant material, it is possible to isolate druggable leads towards treatment of various ailments like infections, inflammations and etc,

Keywords: *Acacia auriculiformis*, Phytochemical Screening, Antioxidant, DPPH assay, Anti-inflammatory activity, and FTIR Analysis.

1. Introduction

Phytochemicals of medicinal plants are the origin of medicines for healing various health disorders and the potential sources of new drug research and development [1]. The most accessible and easily affordable medicines for primary health care in developing countries remain medicinal plant products. The phytochemistry of plants has the primary advantage of applying plant products as a potential remedy to different ailments. Because of their minimal side effects, plant-based medicines are the primary remedy to treat different human and live-stock ailments and still have a high acceptance level in the community [2].

Medicinal plants have been discovered for medicinal purposes since prehistoric times. Plants synthesize hundreds of chemical compounds including defense against harmful organisms [3]. Numerous phytochemicals with potential biological activity are identified. However since a whole plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain [4]. Phytochemical effects and pharmaceutical activities of many medicinal plants remain unassessed by rigorous scientific research to define efficacy and safety [5].

Acacia auriculiformis, commonly known as auri, earleaf acacia, ear pod wattle, northern black wattle, Papuan wattle, and tan wattle, akashmoni in Bengali, is a fast- growing, crooked, gnarly tree in the family Fabaceae. It is native to Australia, Indonesia, and Papua New Guinea [6]. *Acacia auriculiformis* A.Cunn. ex Benth. is a perennial shrub having a wide range of medicinal potentials and is widely distributed throughout the world. It is being used traditionally to overcome various medical complications like sore eyes, aches, rheumatism, allergy, itching, and rashes [7].

Acacia auriculiformis tree is used as a folk medicine by the natives for various diseases. For the treatment of sore eyes and aches, a decoction of the root is used for rheumatism treatment and bark infusion is used by the Australian aborigines [8]. The various parts of *A. auriculiformis* plant extract and phytoconstituents are found to be useful in various diseases like candidiasis, rheumatism, conjunctivitis, pain, anthelmintic, human immunodeficiency virus (HIV), and microbial diseases [9]. Besides, Acacia auriculiformis has been proven for many pharmacological activities like central nervous system depressant activity, antimicrobial, antimalarial, anti-filarial, cestocidal, antimutagenic, chemopreventive, spermicidal, wound healing, hepatoprotective and antidiabetic activity due to its low toxicity and high efficacy [10-11]. *Acacia auriculiformis* is a multipurpose shrub which has been widely used as traditional medicine in several Asian countries. This plant has been used to treat several medical ailments due to its low toxicity and the presence of bioactive phytoconstituents [12]. The present work reveals that *A. auriculiformis* is a treasured source of medicinally important molecules and provides an authentic base for its future use in contemporary medicine.

Fig 1: *Acacia auriculiformis* Benth.

2. Materials and Methods

2.1 Plant Collection and Authentication:

Acacia auriculiformis flowers were collected from the Mainpat Hiis Surguja Chhattisgarh. The plants were authenticated by Prof. Rijwan Ulla, Department of Botany, Rajeev Gandhi Govt. Autonomous Post Graduate College Ambikapur, Surguja, Chhattisgarh, India. The plant materials were dried under shade by placing in a single layer and coarsely powdered by hand mixer and pass through sieve no 60.

2.2 Preparation of Plant Extract

The collected samples were washed with clean water and dried in the shed for about three weeks. The dry samples were chopped into pieces and ground into powder by using a mechanical grinder. The powdered materials were stored in clean plastic bottles until the use. The materials were subjected to Soxhlet's extraction using methanol and aqueous solvent. The extracts were concentrated in a rotary evaporator. The extracts were stored at 4˚C, till used for analysis.

2.3 Physicochemical analysis

Physicochemical parameters include loss on drying, water content, total ash content, acid-insoluble ash content, water-soluble ash content, water-soluble extract content, and ethanol-soluble content [13-14].

(i) Determination of loss on drying (LOD)

LOD was determined by gravimetric determination. 2–5 g of sample was placed in crucible porcelain, dried at 105 °C for 60 min, then moved into a desiccator. This process was repeated until the constant weight was achieved. LOD was expressed as gram per gram of air-dried (World Health Organization 1998).

(ii) Determination of water content

Water content was determined by gravimetric method. 1 g of sample was heated in the oven at 105ºC for 5 h, and then weighed. The process was continued with 1 h intervals until the difference between 2 consecutive weighings is not more than 0.25%.

(iii) Determination of total ash content

1 g of sample was placed in a silicate crucible and weighed. Sample was spread in an even layer in the crucible, and the material ignited by gradually increasing the heat to 500–600 °C until free from carbon, cooled in a desiccator, and weighed. Repeatedly until a fixed weight is obtained (World Health Organization 1998).

(iv) Determination of acid-insoluble ash content

Ash obtained from ash content testing was boiled with 25 mL HCl (~70 g/l) TS for 5 min. The ash is filtered with non-ash filter paper and washed with 5 mL hot water. The insoluble matter was transferred to the crucible, dried on a hot-plate and ignited to constant weight, and placed in a desiccator for 30 min, then weighed without delay. Content of acid-insoluble ash is calculated in mg per g of air-dried material (World Health Organization 1998).

(v) Determination of water-soluble ash content

Containers containing total ash were added with 25 mL of water and boiled for 5 min. Material that does not soluble is collected into a glass cup or ashless filter paper. Then, it was washed with hot water and ignited in a cup for 15 min at a temperature of 450 °C until the weight remained. The reduction of the residue weight in mg is total ash weight. The water-soluble ash is calculated in mg per g of air-dried material (World Health Organization 1998).

(vi) Determination of water- and ethanol-soluble extract

5 g of extract was macerated with 100 mL of water for 6 h for water-soluble extract determination, and then saturated with CHCl3. For ethanol-soluble determination, it was macerated with ethanol. They were shaken frequently, and allowed to stand for 18 h. The extract produced was filtered and poured into a volumetric flask. 20 mL of extract was transferred to a porcelain cup,

2.4 Phytochemical analysis:

Each extract was subjected to qualitative tests for identification of various constituents like alkaloids, carbohydrates, glycosides, steroids, saponins, flavonoids, tannins and phenolic compounds and proteins. The preliminary phytochemical screenings of extracts were performed according to standard procedure [15-18]. These extracts were tested for the presence of various bioactive compounds which are given below:-

1 **Alkaloids:** Tests were conducted using precipitation reactions with Mayer's and Wagner's reagents. A total of 1 mL of the extract (or fraction) was divided into two equal parts: one was treated with 0.5 mL of Mayer's reagent and the other with 0.5 mL of Wagner's reagent. The presence of alkaloids was confirmed by the formation of a white precipitate with Mayer's reagent or a brown precipitate with Wagner's reagent.

2 **Tannins:** Tannins were detected by adding 1 mL of water and 1 to 2 drops of a 1% $FeCl_3$ solution to 1 mL of the extract (or fraction). The appearance of a dark green or blue-green color indicated the presence of tannins. Dark green suggested catechin tannins, while blue-green indicated the presence of gallic tannins.

3 **Flavonoids:** A total of 1 mL of the extract (or fraction) was placed in a test tube. To this, 1 mL of hydrochloric acid (HCl) and three magnesium chips were added. A red or yellow coloration revealed the presence of flavonoids.

4 **Saponins:** A total of 10 mL of the hexanoic extract was placed in a test tube, shaken for a few seconds, and left to stand for 15 min. The formation of a persistent foam indicated the presence of saponins.

5 **Terpenoids:** A total of 5 mL of the extract was added to 2 mL of chloroform and 3 mL of concentrated sulfuric acid (H2SO4). The formation of two phases and a brown color at the interface indicated the presence of terpenoids.

6 **Glycosides: Legal test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

7 **Reducing Sugars:** To the test solution, 2ml of Fehling's reagent was added followed by 3ml of water, formation of Red-Orange color showed the presence of reducing sugars.

8 **Anthraquinones:** Five ml of the extract solution was hydrolyzed with conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

9 **Phenolic compound:** A small amount of the plant extract is dissolved in distilled water. A few drops of 5% or 10% neutral ferric chloride solution are added to the solution. A color change to blue, green, or purple indicates the presence of phenolic compounds.

10 **Proteins and Amino acids:** (a) Biuret test**:** Added 1ml of 40% sodium hydroxide solution and 2 drops of 1% $CuSO_4$ solution till a blue color was produced, and then added to the 1ml of the extract. Formation of pinkish or purple violet color indicated the presence of proteins.

(b) Ninhydrin test: Added two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

2.5 Quantitative Phytochemical Screening

The quantitative phytochemical screening was performed by determining total phenolic content (TPC) and total flavonoid content (TFC) of the extracts. The TPC and TFC of the extracts were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of extracts and milligrams quercetin equivalent per gram (mg QE/g) of extracts, respectively.

2.5.1 Determination of Total Flavonoid Content (TFC)

0.5 ml of each extract (50 mg/ml) was separately mixed with 1.5 ml methanol and 0.1 ml aluminium trichloride (10%). Then, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water was added into each test tube. Then, absorbance at 415 nm was measured after it was allowed to stand in the dark for 30 min using a UV-visible spectrophotometer. Finally, a calibration curve was prepared with a series of quercetin standards (10-50 µg/ml) and the total flavonoid compound concentration was determined in terms of mg QE/g of the extract [19].

2.5.2 Determination of Total Phenolic Content (TPC)

Folin–Ciocalteu reagent was used for the determination of total polyphenolic content. 0.5 ml of each extract (5 mg/ml), Folin–Ciocalteu reagent (5 ml, 1:10 v/v diluted with distilled water) and aqueous sodium carbonate (4 ml, 1 M) solution were mixed together. The mixture was allowed to stand in the dark for 15 min at room temperature, and the absorbance at 765 nm was measured with the help of ultraviolet (UV-visible) spectrophotometer. Then, the total polyphenolic content was determined in terms of mg GAE/g of dry weight of the extract with the help of a calibration curve prepared with a series of gallic acid standards (10-80 µg/ml) [20].

2.6 In-Vitro Antioxidant Activity

Hydrogen-Donating Activity (DPPH free Radical scavenging)

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 colored 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. 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 percentage of antioxidant activity (% AA). Ascorbic acid was used as standard [21-22].

The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation:

$$\text{Antioxidant activity (\%)} = \left(\frac{\text{Abs}_{1h}}{\text{Abs}_{\text{initial}}}\right) \times 100\%$$

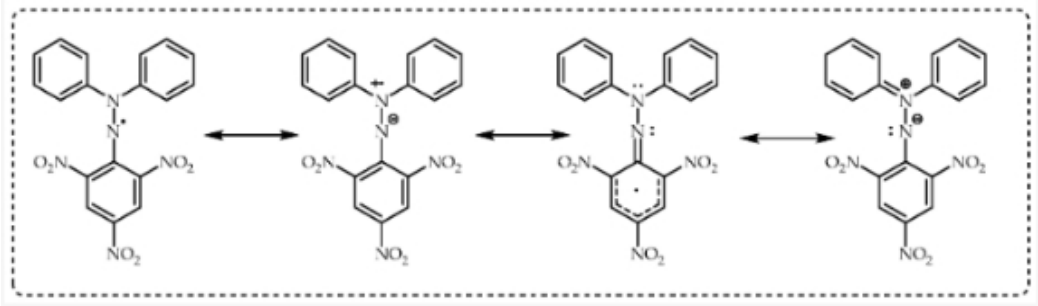


Fig 2. The chemical structures of a 1,1-diphenyl-2-picrylhydrazil radical (DPPH·).

2.7 Assessment of In -vitro anti-inflammatory activity

Inhibition of protein denaturation

The in vitro anti-inflammatory activity of the hydroalcoholic extract of the flower of Acacia auriculiformis was studied using the egg albumin protein denaturation assay according to Fetni et.al with some modifications [23]. For each concentration of methanolic extract (2 mL) or standard (Sodium diclofenac), 2.8 mL of phosphate-buffered saline (PBS), pH= 6.5 was added, mixed with 0.2 mL of egg albumin (fresh). Samples were incubated in a 37 °C oven for 15 min, then immersed in a 70 °C water bath for 5 min. After cooling the tubes, the absorbance (level of protein precipitation) was measured at 660 nm in a spectrophotometer [24].

The percentage inhibition of protein denaturation was calculated as:

Percentage inhibition = (Abs control – Abs sample) × 100/Abs control

The result is an average of three replicates. This method evaluates the potential of the extract to inhibit the denaturation of proteins, hence indicating its anti-inflammatory properties.

2.8 Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan) [25], with a Scan range from 400 to 4000 cm 1 with a resolution of 4 cm^{-1}

3. Results and Discussion

3.1 Physiochemical parameters

Physiochemical parameters of the flower of *Acacia auriculiformis* are tabulated in Table 4.1. Different extracts of the powdered flower were prepared for the study of extractive values. Percentage of extractive values was calculated with reference to the air-dried drug. The results are shown in Table 4.1. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant material can be easily deteriorated due to fungus. The loss on drying at 105 °C in flower was found to be 10.1%. Total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Physiochemical parameters of the flower of *Acacia auriculiformis* are tabulated in Table 1. Studies of physicochemical characterization can serve as a valuable source of information and are

usually applied in judging the purity and quality of the drug. The extractive values give an idea about the chemical constitution of the drug.

Table 1: Physicochemical analysis of flower *Acacia auriculiformis*

S.N	Parameters	Results (%w/w)
1	Total Ash	6.75
2	Acid insoluble Ash	1.60
3	Water -soluble Ash	4.55
4	Water soluble extractive value	21.92
5	Alcohol soluble extractive value	18.70
6.	Ether- soluble extractive value	10.84
7.	Loss on Drying	5.33

In *Acacia auriculiformis* flower methanol extract has the highest solubility of phytochemicals when extracted with alcohol than aqueous extracts tested. Analytical results showed the results are in agreement with *Acacia auriculiformis* flower extracts total ash 6.75, acid insoluble 1.60, water-soluble ash 4.55, water- soluble extractive value 21.92, alcohol soluble extractive value 18.70, Ether - soluble extractive value 10.84and loss of drying (moisture of contents) 5.33.

3.2 Phytochemical Screening

The preliminary phytochemical constituents detected in the flower are known to be beneficial in the treatment of infected diseases recently, a number of studies has been carries out on the various phytochemical of plant across the worlds evolutions of phytochemical such as alkaloids, flavonoids, glycosides and amino acid revealed the presence of most of the *Acacia auriculiformis* flower constituents in polar extracts. In the present investigation flower of ethanolic and aqueous extract of showed the presence of flavonoids derivative, alkaloids, terpenoids, reducing sugars, tannins, saponins, steroids, glycosides, phenolic compound , protein and amino acid. [table.2]. phenols, mainly the type of flavonoids from some medicinal plants are safe and bioactive and have antioxidants, antibacterial and anti-inflammatory properties. Bioactive compound formed in various plant parts possess multiple biological effects on human and non-human biota.

Table 2: Phytochemical present in *Acacia auriculiformis* flowers of methanol and aqueous

S.N	Phytochemical Test	Methanol Extract	Aqueous Extract
1	Carbohydrate	+	+
2	Protein	+	+
3	Alkaloid	+	+
4	Flavonoid	+	+
5	Steroid	+	-
6	Amino Acid	-	+
7	Tannin	+	-
8	Steroidal glycosides	+	-
9	Phenolic compound	+	+
10	Saponins	+	+

(+) :Presence, (-): Absent, +Ve= presence of compound −Ve= compound is not present

Preliminary phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids, flavonoids, phenolic compounds, and saponins in both methanol and aqueous extracts of Acacia auriculiformis flower. Tannins, steroids, and steroidal glycosides were detected only in methanol extract, while amino acids were found only in aqueous extract.

3.3 Estimation of total phenolics and total flavonoids content

The results given in table 3.2 show that the total phenol content and total flavonoid of *Acacia auriculiformis* flowers are methanolic 363.94± 0.17, 325.25 ± 0.14 and aqueous extract 275.20± 0.34, 253.72± 0.25 respectively. The above results showed that aqueous extract contain less phenolic and flavonoids content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents

3.4 Antioxidant Result

DPPH assay

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to non-radical form, DPPH-H. The purple-colored alcoholic solution of DPPH radical changes to yellow in the presence of hydrogen donating antioxidant which could be measured at 517 nm, the activity is expressed as effective concentration IC_{50}, which is the concentration of the sample leading to 50% reduction of the initial DPPH concentration [26-27].

DPPH is 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 stoichometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. As shown in table *Acacia auriculiformis* of methanol and aqueous extracts strongly scavenged DPPH radical with the IC_{50} being 87.25 µg/ml and 125.01 µg/ml respectively. The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 90.53±0.82. (Table 3)

Table 3: Free radical scavenging capacity of methanol extract of *Acacia auriculiformis* flower

Concentration (µg/ml)	DPPH Scavenging %	
	Methanol Extract	Ascorbic Acid
50	37.16±1.15	90.53±0.82
100	52.52±0.32	-
150	67.15±0.84	-
200	82.38±0.42	-
250	94.12±0.65	-
IC_{50}	87.25	-

Values are mean ± SEM of three determinations

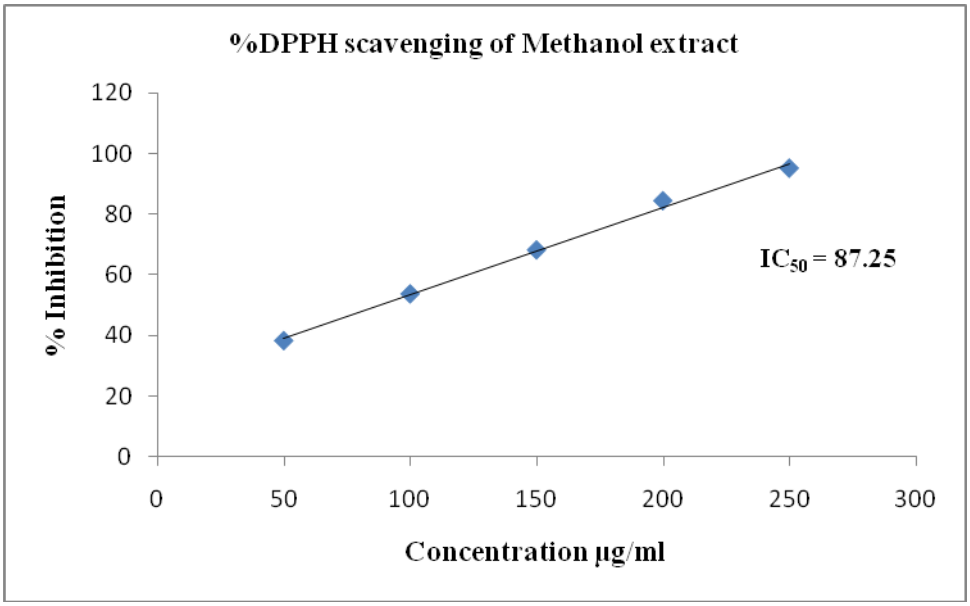
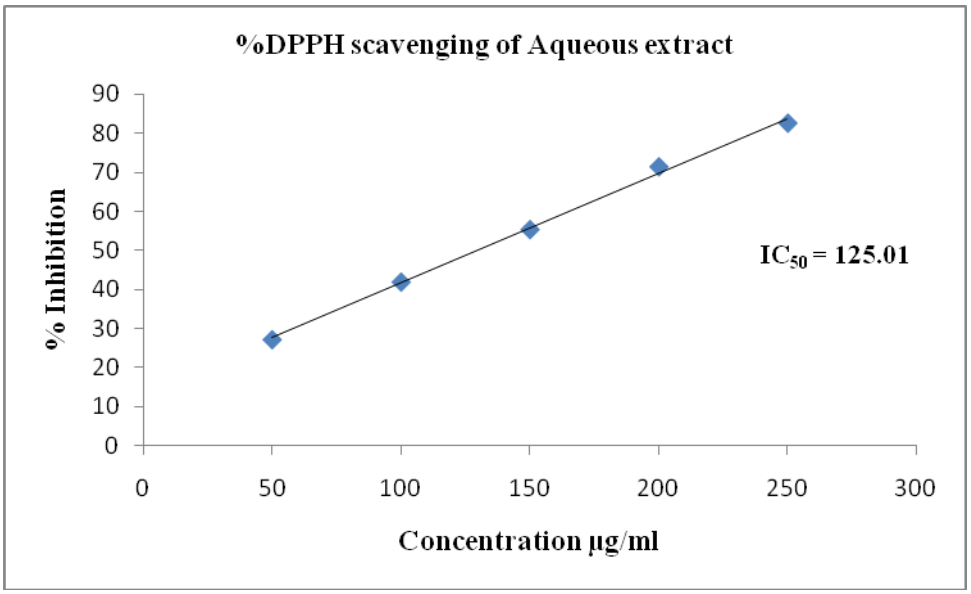


Table 4: Free radical scavenging capacity of aqueous extract of Acacia auriculiformis flower

Concentration (µg/ml)	DPPH Scavenging %	
	Aqueous Extract	Ascorbic Acid
50	28.14±0.35	90.53±0.82
100	42.75±0.70	-
150	56.25±0.48	-
200	72.40±0.30	-
250	83.45±0.63	-
IC_{50}	125.01	-

Values are mean ± SEM of three determinations



Plants are the greatest source of natural antioxidants due to the presence of various biophenolic compounds like phenolic acids, saponins, flavonoids, and tocopherols . Plant materials which are rich in phenol contents, are widely used as medicinal remedies due to their various pharmacological properties [28]. Flavonoids are naturally-occurring compound of plants and account for more than half of the eight thousand different phenolic compounds [29]. They have been shown to effectively scavenge most oxidizing molecules, which include singlet oxygen and other free radicals [30].

3.5 The anti-inflammation activity

The ethical challenges and the nonexistence of rationale to use animals for pharmacological research of new chemical compounds, when other suitable methods are available or could be investigated, pushed us to select the protein denaturation bioassay and membrane stabilization potential for in vitro evaluation of anti-inflammatory property activity of studied plant materials.

The Inhibition of Protein denaturation

Denaturation of tissue proteins is well-documented as one of the causes of inflammatory and arthritic diseases, through auto antigen production [31] and the ability of plant material to bring down the protein denaturation could be effective against inflammation diseases. The in vitro anti-inflammatory activity of the methanolic and aqueous extract of *Acacia auriculiformis* flower and sodium diclofenac was analyzed using the protein denaturation method . Table 5 presents the percentage inhibition of protein denaturation by methanolic and aqueous extracts of *Acacia auriculiformis* flower, compared with the standard drug Sodium Diclofenac at concentrations ranging from 20 to 100 g/L. The protein denaturation assay is used to evaluate anti-inflammatory activity. The results show a concentration-dependent increase in inhibition for all three samples. The methanolic extract exhibited higher activity than the aqueous extract at every concentration. At 100 g/L, the methanolic extract showed `86.70 ± 0.9%` inhibition, while the aqueous extract showed `65.45 ± 0.3%`. Sodium Diclofenac,a standard NSAID drug used as the positive control, demonstrated the highest activity, reaching `120.40 ± 0.6%` at 100 g/L.

This indicates that methanol is more effective in extracting bioactive compounds responsible for anti-inflammatory effects. Although the plant extracts were less potent than the standard drug, the methanolic extract showed significant activity. Overall, the findings support the potential anti-inflammatory use of *Acacia auriculiformis* flowers, particularly in methanolic extract.

Table 5: Percentages of inhibition of protein denaturation of the methanolic and aqueous extract of *Acacia auriculiformis* flower and sodium diclofenac at different concentrations.

S.N.	Concentrations (g/L)	Inhibition of protein denaturation (%)		
		Methanolic extract (%)	Aqueous Extract	Sodium Diclofenac (%)
1	20	12.6 ± 0.5	8.44 ± 0.2	16.8 ± 0.3
2	40	18.70 ± 0.6	13.50 ± 0.5	24.30 ± 0.4
3	60	46. 60 ±1.2	35.70 ± 0.4	68.80 ± 0.8
4	80	64.60 ± 0.3	48.85 ±0.6	98.10 ± 0.2
5	100	86.70 ± 0.9	65.45 ± 0.3	120.40 ± 0.6

Samples and positive control were performed in triplicate (n=3). SD = standard deviation.

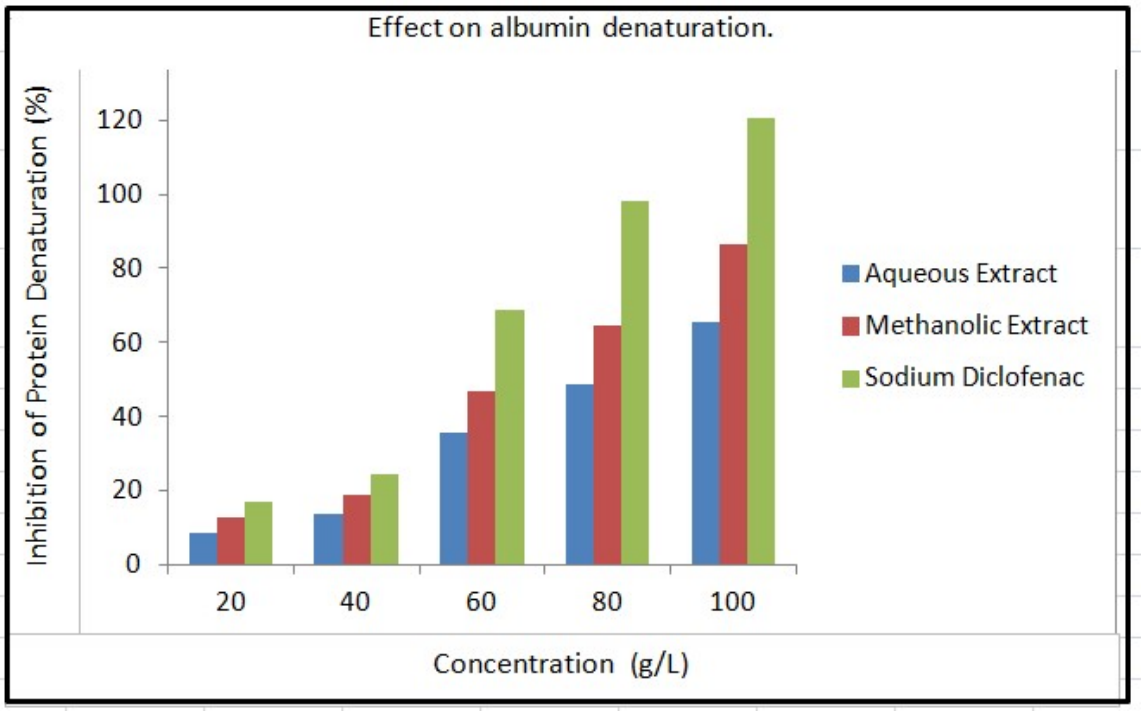


Fig.3: *Acacia auriculiformis* methanol extract effect on albumin denaturation.

This observation is consistent with recent research highlighting the efficacy of plant extracts, particularly those containing flavonoids and saponins, in modulating inflammatory responses. For example, studies have shown that flavonoids found in Acacia auriculiformis flower exert anti-inflammatory effects by inhibiting the release of pro-inflammatory cytokines and modulating cellular pathways involved in inflammation [32].In addition, saponins are recognized for their ability to interfere with the activation of inflammatory mediators, reinforcing the idea that plant extracts may provide a viable alternative to conventional pharmaceutical treatments [33].

Further research highlights the potential of natural products in the treatment of chronic inflammatory diseases, which is particularly relevant given the increasing prevalence of these conditions. The side effects associated with NSAIDs, including gastrointestinal disturbances and cardiovascular risks, underscore the need to seek safer and more effective alternatives. Licorice extract, with its favorable safety profile and demonstrated efficacy, may represent a promising avenue for the development of anti-inflammatory treatments.

3.6 FTIR Spectral Data Interpretation

FT-IR study Table: 6 shows the functional groups of the organic and inorganic compounds of the plant extract. The FTIR methods was performed on a spectrophotometer system, [34], which was used to detect the characteristics peak values and their functional group. Methanolic extract shows strong phenolic and flavonoid signatures. Multiple O-H bands confirm high phenolic content. 1635.44 cm^{-1} suggests conjugated systems typical of flavonoids. 2460.31 cm^{-1} indicates carboxylic acids. Fingerprint region confirms aromatic compounds. Aqueous extract is rich in polar compounds. Strong H-bonded O-H bands + 1750.63 cm^{-1} C=O indicate tannins, glycosides, and organic acids. 1543.78 cm^{-1} N-H bending suggests proteins. Lower fingerprint peaks confirm aromatic glycosides. FT-IR analysis of methanol flower and aqueous extracts of *Acacia auriculiformis.* Flower confirmed the presence of phenols, alcohols, carboxylic acid, amide, aldehydes, ketones, alkanes, alkenes, aromatics, amines and alkyl halides which show major peaks.

Table 6 : FT-IR spectrum of Methanolic and Aqueous extract of *Acacia auriculiformis* Flower

Extract	Peak Value (cm^{-1})	Group	Class
Methanolic Extract	670.2-398.12	C-H bending	Aromatic substitution
	1635.44	C=O strtching /C=C stretching	Carbonyl group aromatic group
	2460.31	O-H stretching	carboxylic acid
	3328.16	O-H stretching	H-bonded phenolic group
	3462.77	O-H stretching	free O-H alcohols and phenol
	3680.45	O-H stretching	free O-H alcohols and phenol
	3694.77	O-H stretching	free O-H alcohols and phenol
Aqueous Extract	423.9-498.36	C-C bending	Aromatic ring
	1429.21	C-H bending/ O-H bending	Alkanes
	1543.78	N-H bending	Amide group
	1635.44	C=C strtching	Conjugated
	1750.63	C=O stretching	C=O group carboxylic acid/ ester
	3328.16	O-H stretching , H bond	Phenols
	3442.72	O-H stretching ,H bond	Phenols
	3648.94	O-H stretching ,H free	Alcohols and Phenol
	3740.51	O-H stretching ,H free	Alcohols and Phenol

Spectral differences are the objective reflection of componential differences. By using FT-IR spectrum, we can confirm the functional constituent's presence in the given parts and extract, identify the medicinal materials from the adulterate and even evaluate the qualities of medicinal materials The results of the present study coincided with the previous observations observed by various plant biologist and taxonomist many researchers applied the FT-IR spectrum as a tool for distinguishing closely associated plants.

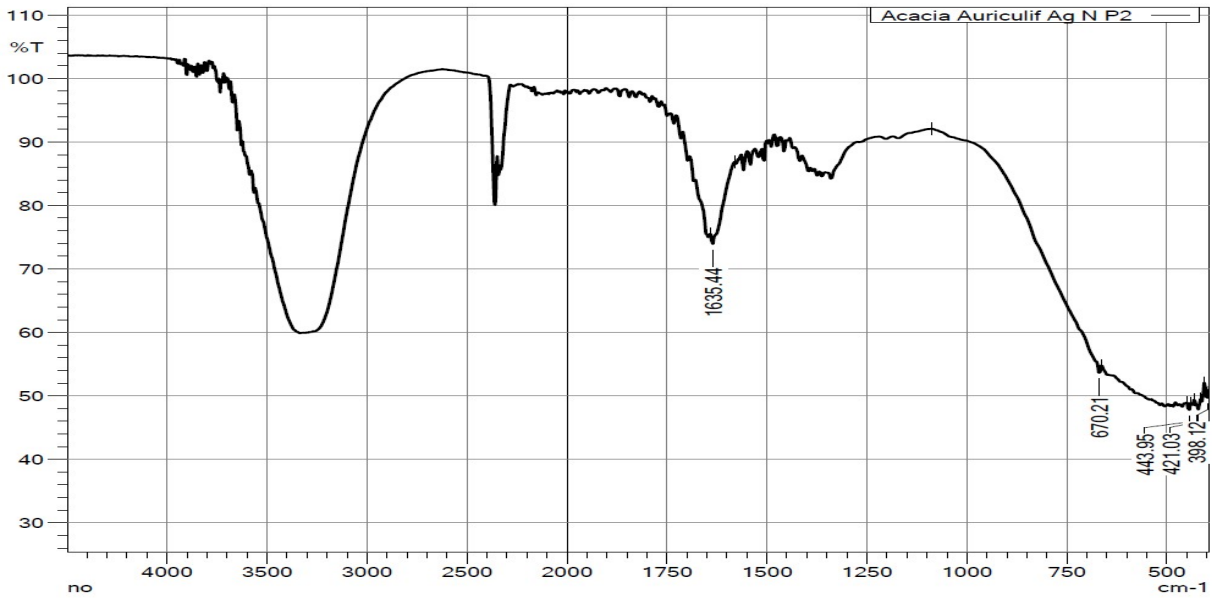


Fig 4: Shows the FT-IR frequency range of methanolic *Acacia auriculiformis* Flower Extract

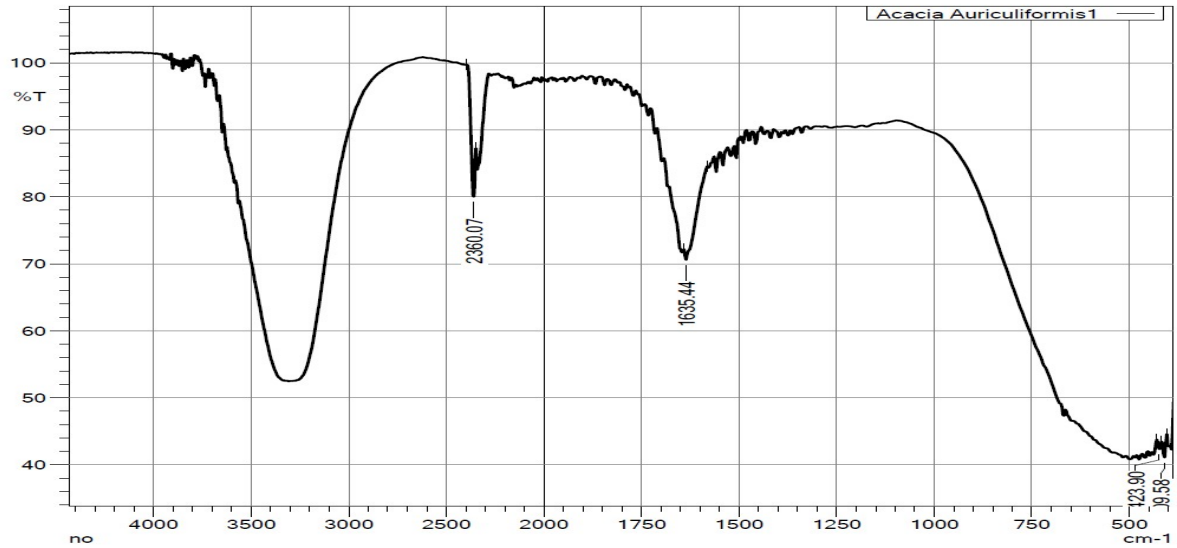


Fig 5: Shows the FT-IR frequency range of aqueous *Acacia auriculiformis* Flower Extract

4. Conclusion

The plant has to be exploited more in the future for its other pharmacological properties. Whole depiction of the current study specified that both aqueous and methanol extracts exhibited high potential of anti- inflammatory and antioxidant activities. The study validates the therapeutic potential of *Acacia auriculiformis* signifying that the plant possibly will "lead" for the separation of new compounds with good competence to treat various diseases and disorders. The outcomes of the present study confirmed the use of this plant in traditional claims in managing diabetics and infections. Novel antibiotics and anti-diabetic drugs could be formulated from *Acacia auriculiformis* by further isolation and purification of active compounds for phytotherapy.

5. Conflicts of interest

The authors declare no conflict of interest.

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References

1. Bachheti, A., Sharma, A., Bachheti, R.K., Husen, A., Pandey, D.P., 2020. Plant Allelochemicals and Their Various Applications. In Co-Evolution of Secondary Metabolites; M´erillon, J.M., Ramawat, K.G., Eds.; Springer International Publishing: Cham, 441–465.
2. Gumisiriza, H., Birungi, G., Olet, E.A., Sesaazi, C.D., 2019. Medicinal Plant Species Used by Local Communities around Queen Elizabeth National Park, Maramagambo Central Forest Reserve and Ihimbo Central Forest Reserve, South Western Uganda. J. Ethnopharmacol. 239, 1–22.
3. Ashok, J.P., Harish, P.H., Prasad, W.V., Ashok, W.A., 2018. Comparative assessment of antioxidant potential of *A. auriculata* flower, leaf and seed methanolic extracts. Int. J. Pharm. Pharm. Sci. 7, 381-385.
4. Bora, R., Khakhalar, S., Dutta, T., 2019. Phytochemical profiling, assessment of total henolic content, total flavonoid content, and antioxidant activity of ethno- medicinal plant,

Meyna spinosa from Assam. Asian J. Pharm. Clin. Res. 12, 61- 63.
5. Chopra RN, Nayar SL and Chopra IC. (1956). Glossary of Indian Medicinal Plants. CSIR PID, New Delhi, India.
6. Subhan, N., Burrows, G. E., Kerr, P. G., and Obied, H. K. 2018. Phytochemistry, Ethnomedicine, and Pharmacology of Acacia. Studies in Natural Products Chemistry Elsevier B.V
7. Girijashankar V. Micropropagation of multipurpose medicinal tree *Acacia auriculiformis*. J Med Plants Res 2011; 5(3): 462-466.
8. Sharma N, Singh S, Singh SK. Pharmacognostical standardization and preliminary phytochemical investigations on *Acacia auriculiformis* Cunn. Ex. Benth stem bark. J Med Plants Res 2017; 5(1): 398-902.
9. Sravanthi, S., Santosh, C., & Mohan, M. M. (2014). Phytochemical analysis, antioxidant and antimicrobial activities of ethanolic extract of *Acacia auriculiformis.* Journal of Environmental and applied Bioresourse, 2(1) 1-4.
10. Prayogo, Y. H., Wahyudi, S. T., Batubara, I., Sari, R. K., & Syafii, W. (2024). Multitarget ensemble docking of potent anticancer and antioxidant active compounds from *Acacia auriculiformis* and *Acacia crassicarpa*. Research Journal of Pharmacy and Technology, 17(2), 707-716.
11. Adebayo, S.A., Dzoyem, J.P., Shai, L.J., Eloff, J.N., 2015. The Anti-Inflammatory and Antioxidant Activity of 25 Plant Species Used Traditionally to Treat Pain in Southern African. BMC Complement. Altern. Med. 15, 1–10
12. Singh, R., Singh, S., Kumar, S., & Arora, S. (2007). Free radical-scavenging activity of acetone extract/fractions of *Acacia auriculiformis* A. Cunn. Food Chemistry, 103(4), 1403-1410.
13. USP (2015). United States Pharmacopoeia Drug Information for the Health Care Professional. Thomas PDR. Micromedex. 1.
14. Mukherjee PK. Quality control herbal drugs: An Approach to Evaluation of Botanicals,4th edition, Business Horizons Pharmaceutical Publishers, New Delhi, 2008, 183-195.
15. World Health Organization (WHO). Quality Control Methods for Medicinal Plant Materials. WHO/PHARM/92.559. Geneva: World Health Organization (WHO); 1998. p. 4-46/
16. Trease GE, Evans WC. Treas and Evans Pharmacognosy. (14th ed) India. W.C. Saunders company limited, 1989.
17. Sofowora A. Medicinal and Traditional medicin in Africa. Ibadan Nigeria spectrum books Limited. 1998, 130.
18. Harbone, J.B. (1984). Phytochemical methods: A Guide to Modern Techniques of Plant Analysis. 2nd ed. Chapman and Hall Ltd, New York, p. 37-38.
19. Rohit Kumar Bargah, Pradeep Singh Painkra, Prabha Wati Painkra, Ankit Kumar Soni, Sanjeeta Tirkey, Harawati Painkra and Punita Bhagat. Qualitative and quantitative phytochemical analysis and in vitro antioxidant activity of flowers extract from *Gmelina arborea* Roxb. (Verbenaceae). Int. J. Chem. Res. Dev. 2024; 6(2):68-73.
20. Pandey, S., Satpathy, G., & Gupta, R. K. (2014). Evaluation of nutritional, phytochemical, antioxidant, and antibacterial activity of exotic fruit *Limonia acidissima*. Journal of Pharmacognosy and Phytotherapy, 3(2), 81-88.
21. Nivetha, K., & Prasanna, G. (2016). GC-MS and FT-IR analysis of Nigella sativa L. seeds. International Journal of Advanced Research in Biological Sciences, 3(6), 45-54.
22. Singh, R., Singh, S., Kumar, S., & Arora, S. (2007). Studies on antioxidant potential of methanol extract/fractions of *Acacia auriculiformis* A. Cunn. Food Chemistry, 103(2), 505-511.
23. Heeralal Singh, Arun Kumar Kashyap and Rohit Kumar Bargah (2026); Phytochemical, antioxidant and antidiabetic effect of methanolic extract of *Senna surattensis* flower, Journal of Pharmacognosy and Phytochemistry 2026; 15(3): 820.827.
24. Rohit Kumar Bargah, Naveen Kumar, Prakash Singh, Sandeep Kujur, Priyanka Painkra, Gulnaz Shahin. (2025).

Phytochemical Screening, Biogenic Synthesis, Characterisation and Antibacterial activity Investigation of Zinc oxide nanoparticles (ZnO -NPs) Using *Biancaea decapetala* Leaf extract. Int. J. Curr. Res. Chem. Pharm. Sci. 12(7): 1-17.

25. Rohit Kumar Bargah, Pradeep Singh Painkra, Prabha Wati Painkra, Ankit Kumar Soni, Sanjeeta Tirkey, Harawati Painkra and Punita Bhagat. Qualitative and quantitative phytochemical analysis and in vitro antioxidant activity of flowers extract from *Gmelina arborea* Roxb. (Verbenaceae). Int. J. Chem. Res. Dev. 2024;6(2):68-73. DOI: 10.33545/26646552.2024.v6.i2a.68
26. Bargah, R.K. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. J Pharmacogn Phytochem 2015; 4(1):07-09.
27. Bargah, R.K., (2017), Preliminary Phytochemical Screening Analysis and Therapeutic Potential of *Tecoma stans* (L.), International Journal of Applied Chemistry. Vol. 13(1) , pp. 129-134.
28. Rohit Kumar Bargah, Anil Kushwaha, Arun Tirkey, Bimlesh Hariwanshi In Vitro Antioxidant and Antibacterial Screening of flowers Extract from *Cassia auriculata* Linn. Research J. Pharm. and Tech 2020; 13(6):2624-2628.
29. Zheng W, Wang SY. (2001). Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 49:5165- 5170.
30. Olayinka A, Aiygoro and Anthony Okoh. (2010). Priliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC, 10:21
31. Gandhidasan R, Thamaraichelvan A, Baburaj S. Anti inflammatory action of Lannea coromandelica by HRBC membrane stabilization. Fitoterapia 1991; Voll LXII; No1; 81- 83.
32. Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. Evaluation of antiinflammatory activity of *Tephrosia purpurea* in rats. Asian Pac J Trop Med 2010; 3(3): 193-5.
33. Rajendran Vadivu, Lakshmi K S. In vitro and in vivo anti inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssp Laurina. Bangladesh J Pharmacol 2008; 3: 121-124.
34. Nivetha, K., & Prasanna, G. (2016). GC-MS and FT-IR analysis of *Nigella sativa* L. seeds. International Journal of Advanced Research in Biological Sciences, 3(6), 45-54.

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