EXTRACTION, PHYTOCHEMICAL AND ANTI-OXIDANT EVALUATION OF *LUDWIGIA ADSCENDENS, LAUNAEA PINNATIFIDA* AND *CARICA PAPAYA*

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ABSTRACT

This study investigates the extraction, phytochemical composition, and antioxidant activity of *Ludwigia adscendens, Launaea pinnatifida,* and *Carica papaya* to evaluate their potential for medicinal applications. Hydroalcoholic extracts of the plant leaves were obtained using Soxhlet extraction, followed by qualitative and quantitative phytochemical analysis. Key bioactive constituents including flavonoids, phenolics, alkaloids, and terpenoids were identified. The antioxidant potential was assessed through DPPH, reducing power, and hydrogen peroxide scavenging assays. Among the three species, *Carica papaya* demonstrated the highest total phenolic (124.74 mg GAE/g) and flavonoid content (105.53 mg RE/g), along with the strongest radical scavenging activity (DPPH inhibition: 86.77%; IC_{50}: 22.09 µg/mL). *Ludwigia adscendens* and *Launaea pinnatifida* also exhibited moderate antioxidant efficacy. These findings suggest that all three plants, particularly *Carica papaya*, possess significant antioxidant properties due to the presence of phytochemicals and may serve as promising sources for natural therapeutic agents. Further pharmacological studies are warranted to explore their clinical relevance and bioactive compound mechanisms.

Keywords: Phytochemicals, Antioxidant activity, *Ludwigia adscendens*, *Launaea pinnatifida*, *Carica papaya*, Medicinal plants.

1. INTRODUCTION

Traditionally, plants are viewed as the key source of medications and have a significant part in the general well-being of the community. Plant secondary metabolites have long been

recognized for their biological impacts on humans. Various plant components, like leaves and bark, fruits, flowers, and roots, are recognized to have major medicinal qualities due to availability of a variety of bioactive byproducts. Ornamental plants often grown for aesthetic purposes in home gardens[1]. Medicinal plants have been widely recognized for their therapeutic potential because of the existence of bioactive substances that have pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, along anticancer activities[2, 3]. The use of plant-based antioxidants has gained significant attention in recent years as they help in neutralizing free radicals, reducing oxidative stress, and preventing chronic diseases such as cardiovascular disorders, diabetes, and neurodegenerative conditions[4, 5]. Among various medicinal plants, *Ludwigia adscendens*, *Launaea pinnatifida*, and *Carica papaya* have been reported to have different array of phytoconstituents, such as flavonoids, phenolics, alkaloids, tannins, and terpenoids, which contribute to their pharmacological activities[6]. *Ludwigia adscendens*, commonly known as creeping primrose-willow, belongs to the family Onagraceae and is extensively dispersed in wetland ecosystems of tropical and subtropical regions[7]. It is traditionally utilized in traditional medicine to treat inflammatory disorders, liver ailments, and gastrointestinal complications[8]. The plant is abundant in bioactive substances that have been shown to have hepatoprotective and antioxidant qualities, including flavonoids, tannins, and polyphenols[9]. According to studies, *L. adscendens's* capacity to scavenge free radicals and alter oxidative stress pathways is largely responsible for its antioxidant activity. *Launaea pinnatifida,* One of the participants in the Asteraceae family, is halophytic medicinal plant found around coastal regions and has been traditionally used in Ayurveda for treating gastrointestinal disorders, inflammation, and hepatic diseases[10, 11]. The plant is known to be a plentiful supply of phytoconstituents like flavonoids, phenolics, and sterols, which contribute to its antioxidant and hepatoprotective activities[12]. Several studies have demonstrated the capacity to scavenge free radicals *L. pinnatifida* extracts, supporting its role as a natural antioxidant in preventing oxidative damage[13, 14]**.** The presence of polyphenols in *L. pinnatifida* has also been linked to its anti-inflammatory and antimicrobial properties. *Carica papaya* (Caricaceae), commonly called as papaya is a tropical fruit-bearing plant widely cultivated for its nutritional and medicinal benefits[15]. Different portions of *C. papaya*, collectively leaves, seeds, and unripe fruits, have been extensively studied for their therapeutic properties, particularly their antioxidant, antimicrobial, and anticancer activities [16, 17]. The plant contains a significant amount of bioactive materials like alkaloids, flavonoids, tannins, carotenoids, and vitamins that contribute to its strong antioxidant potential[18, 19]. According to studies, papaya extracts have

strong free radical scavenging properties that lessen the problems associated with oxidative stress. The current study intends to examine the extraction, phytochemical content, and antioxidant evaluation of *Ludwigia adscendens*, *Launaea pinnatifida*, and *Carica papaya* in light of the growing interest in natural antioxidants. The study will provide valuable insights into their potential applications in nutraceuticals, pharmaceuticals, and functional food industries[20, 21]. By identifying and characterizing the bioactive compounds responsible for their antioxidant properties, this research seeks to contribute to the growing field of plant-based therapeutics and support the development of natural antioxidant formulations[22,23].

2. RESOURCES AND PROCEDURES

2.1 Plant material collection

From the vicinity of Bhopal, 100 grams of the medicinal plants *Ludwigia adscendens*, *Launaea pinnatifida*, and *Carica papaya* were arranged. Following cleaning, plant parts (leaves) were baked at 45°C until they were completely dry after three days of room temperature drying in the shade. To prevent contamination and deterioration, dried plant components were kept in cold, dry environments in airtight glass containers. Medicinal plants *Ludwigia adscendens, Launaea pinnatifida, and Carica papaya* were authenticated by a plant taxonomist on September 26, 2023, from Govt. College Khimlasa, Sagar, (M.P.) under Reference nos. 2023071, 2023072, and 2023073, respectively, to verify their identity and purity.

2.2 Soxhlet extraction:

After being dried and ground into a powder using petroleum ether, the leaves of Ludwigia adscendens, Launaea pinnatifida, and Carica papaya were put in a Soxhlet apparatus thimble. Using a hydroalcoholic solvent system, the extraction was done for 8–10 hours at a temperature of 40–60 degrees Celsius in the heating mantle. Following the extraction procedure, the sample extracts were filtered and dried out. Airtight containers were used to collect the extracts[24,25]. Below equation was made to count extraction yields for each extract:

$$\text{Formula of \% yield} = \frac{\text{Original yield}}{\text{Yield in theory}} \times 100$$

2.2 Evaluation of Extracts Using Qualitative Phytochemical Methods

Extensive phytochemical analysis was conducted to determine whether certain phytoconstituents were present in petroleum ether and hydroalcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya* using standard procedures [26, 27].

2.3 Quantitative assessment of phytochemicals -

2.3.1 Measurement of TPC: -

TPC of plant extracts was assessed using the Folin-Ciocalteu Assay. The *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* hydroalcoholic Extracts (0.2 mL from stock solution) was mixed with 2.5 ml of Folin-Ciocalteu's phenol reagent individually. After 5 min, 2 ml of a 7.5% Na_2CO_3 solution was added to the mixture followed by the addition of 7 ml of deionized distilled water and mixed thoroughly. The absorbance at 760 nanometer was quantified following mixture's 90-minute dark storage at 25°C. The TPC was calculated by extrapolating the calibration curve, which was created making solution of gallic acid (20 to 100 µg/ml). Three separate measurements of the phenolic chemicals were made. In milligrammes of gallic acid equivalents (GAE) per gramme of dry mass, the TPC value was stated. [28].

2.3.2 Quantification of TFC by Spectrophotometry: -

The content of flavonoids used the aluminium chloride to quantify approach[29]. 0.15 millilitres AlCl3.6H2O (10%) and NaNO2 (5%) were combined with 0.5 ml of each of the hydroalcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida,* and *Carica papaya* in a 10-milliliter test tube. One milliliter of 4% NaOH was added after five minutes. Before the solution had been well mixed, absorbance at 510 nanometer was quantified and compared to the reagent blank. The rutin standard solution (20 to 100µg/ml) was utilised to generate the standard curve for total flavonoid utilising the same process as previously explained. TFC was articulated in mg of rutin comparables per gm of dried fraction[30].

2.4 Activity of antioxidants *in vitro*

2.4.1 Radical Scavenging Activity of DPPH

For the investigation, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was prepared in a 0.1 mM solution in methanol. Different amounts of excerpts and the norm were collected from the solution in stock and combined with 2 milliliters of DPPH reagent to create a 1 mg/ml of the extracts with a typical solution of methanol. At 517 nm, the absorbance was assessed having

half-hour cultivation time in the dark. To establish a control, 3 millilitres of 0.1 mM DPPH solution were added, which was then incubated for 30 minutes[31]. % of antioxidant activity in sample or standard was computed using the following formula:

Percentage of Inhibition = [(Ab of control-Ab of sample/Ab of control x 100].

2.4.2 Assay of reducing power

Three milligrams ascorbic acid is mixed in pure water or a solvent to create standard solutions, which are then diluted to yield concentrations of 20, 40, 60, 80, and 100 µg/ml. One milligram dried excerpt is dissolved in one milliliter of methanol to create the extracts, and sample concentrations are then created. In order to reduce power, aliquots of different ascorbic acid and extract concentrations are mixed with 1.0 ml of deionised water, 2.5 ml of phosphate buffer, and 1 ml of potassium ferricyanide. 20 minutes of incubation at 50°C in a water bath is continued by the addition of 2.5 ml of 10% trichloroacetic acid and a 10-minute centrifugation at 3000 rpm. Utilising a UV spectrophotometer, the absorbance at 700 nm is measured when a blank devoid of extract is created[32].

2.4.3 H2O2 Assay

The approach developed by Ruch et al. used to evaluate the ability of excerpts scavenge hydrogen peroxide (H2O2)[33]. After adding 0.6 mL of H2O2 solution (2 mM) to the 0.1 mL aliquot of extracts (20–100 µg/mL) in the eppendorf tubes, the tubes' capacity was increased to 0.4 mL using 50 mM phosphate buffer (pH 7.4). Following ten minutes of vortexing, the reaction mixture's absorbance was quantified at 230 nm. The positive control in this experiment was ascorbic acid. The ability of the extracts to scavenge H2O2 was calculated using the following formula.

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

3. OUTCOMES

3.1 The yield of the plant extract in percentage

Table 1 Extract yield in percentage

S. No.	Plant name	Solvent	Colour of excerpt	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Ludwigia adscendens*	Petroleum Ether	Dark yellow to brown	100	0.859	0.85
2.	*Ludwigia adscendens*	Hydroalcoholic	Dark brown	99.130	3.102	3.12

3.	*Launaea pinnatifida*	Petroleum Ether	Yellow	100	0.481	0.48
4.	*Launaea pinnatifida*	Hydroalcoholic	Brown	99.498	5.800	5.82
5.	*Carica papaya*	Petroleum Ether	Dark yellow to brown	100	1.101	1.10
6.	*Carica papaya*	Hydroalcoholic	Brown	98.852	8.102	8.19

3.2 Solubility determination of Hydro alcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya*-

Table 2: Finding of Solubility of Hydro alcoholic extracts

S. No.	Solvents	Result		
		Ludwigia adscendens	***Launaea pinnatifida***	***Carica papaya***
1.	Water	Sparingly Dissolvable	In part soluble	In part soluble
2.	Ethanol	Soluble	Partially Dissolvable	Dissolvable
3.	Ethyl Acetate	Sparingly Soluble	Sparingly Soluble	Soluble
4.	DMSO	Soluble	Soluble	Soluble
5.	Petroleum Ether	Insoluble	Insoluble	Partially Soluble
6	Methanol	Freely Soluble	Soluble	Freely Soluble
7	Chloroform	Soluble	Soluble	Soluble
8	Acetone	Dissolvable	Sparingly Dissolvable	Dissolvable

3.3 Analysis of Phyto constituents of various extracts

Table 3 Analysis of phytoconstituents of *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya* extracts

S. No.	Experiment	*Ludwigia adscendens*		*Launaea pinnatifida*		*Carica papaya*	
		Petroleum ether	**Hydroalcoholic**	**Petroleum ether**	**Hydroalcoholic**	**Petroleum ether**	**Hydroalcoholic**
Test for Carbohydrates							
1.	Molisch's Test	-	+	-	+	+	+
2.	Fehling's Test	-	+	-	-	-	+

3.	Benedict's Test	+	+	+	+	+	+
4.	Bareford's Test	-	-	-	+	-	+
Test for Alkaloids							
1.	Mayer's Test	+	+	+	+	+	+
2.	Hager's Test	-	+	-	-	-	+
3.	Wagner's Test	-	+	-	+	-	+
Test for Terpenoids							
1.	Salkowski Test	+	+	-	+	+	+
2.	Libermann-Burchard's Test	-	-	-	-	-	+
Test for Flavonoids							
1.	Lead Acetate Test	-	+	-	+	-	+
2.	Alkaline Reagent Test	-	+	-	+	-	+
Test for Tannins and Phenolic Compounds							
1.	$FeCl_3$ Test	-	+	+	+	-	+
2.	Lead Acetate Test	-	+	-	+	-	+
3.	Gelatine Test	-	-	-	+	-	+
Test for Saponins							
1.	Froth Test	+	+	+	+	+	+
Test for Protein and Amino acids							
1.	Ninhydrin Test	-	+	-	-	-	+
2.	Biuret's Test	-	+	-	+	-	+
Test for Glycosides							
1.	Legal's Test	-	-	-	+	-	+

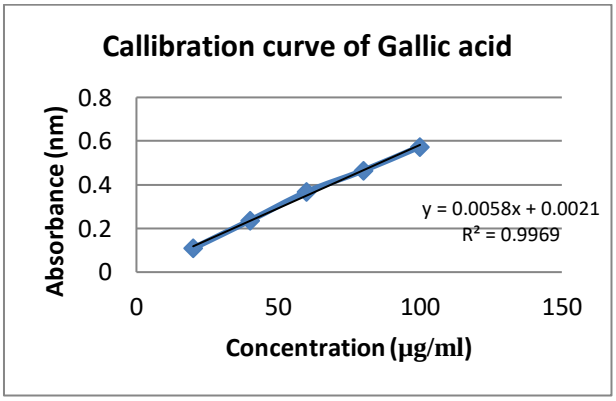
2.	Keller Killani Test	-	+	-	+	+	+
3.	Borntrager's Test	-	+	-	+	-	+

3.4 Quantitative analysis of phytochemicals of Hydroalcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya* -

3.4.1 Estimation of Total Phenolic Content (TPC)

Table 4: Gallic acid standard table

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.109
2.	40	0.236
3.	60	0.367
4.	80	0.465
5.	100	0.575



Graph 1: The graph displays the gallic acid standard curve.

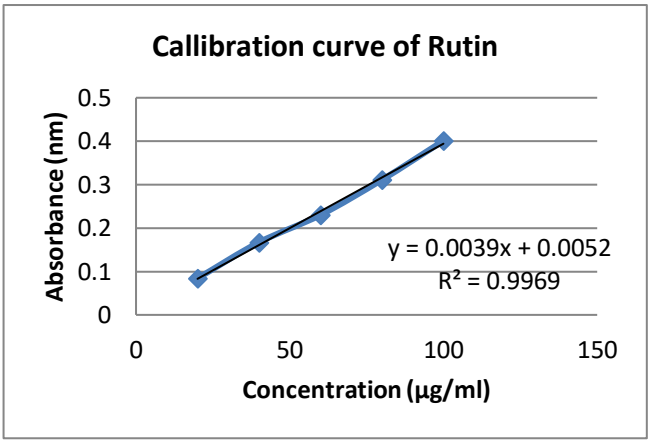
Table 5: Total Phenolic Content in *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* Hydro alcoholic Exerupt-

Phenolic content overall (mg/gm = gallic acid)			
Extracts	***Ludwigia adscendens***	***Launaea pinnatifida***	***Carica papaya***
Absorbance Mean±SD	0.4563±0.003	0.1951±0.002	0.6257±0.001
TPC	90.86	38.62	124.74

3.4.2 Estimation of TFC:

Table 6: Table representing the standard values of Rutin

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.084
2.	40	0.167
3.	60	0.23
4.	80	0.311
5.	100	0.401



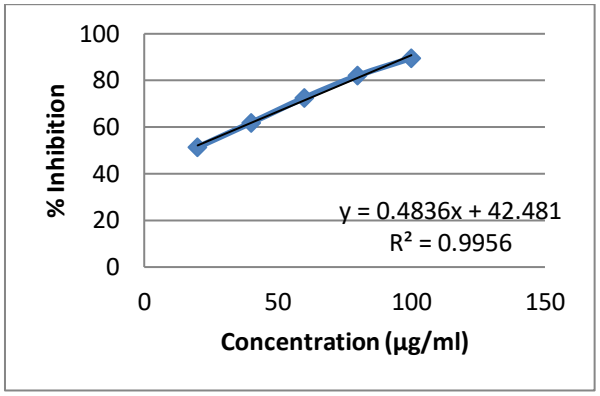
Graph 2: The graph displays the Rutin standard curve.

Table 6: TFC in *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* Hydro alcoholic Excerpt

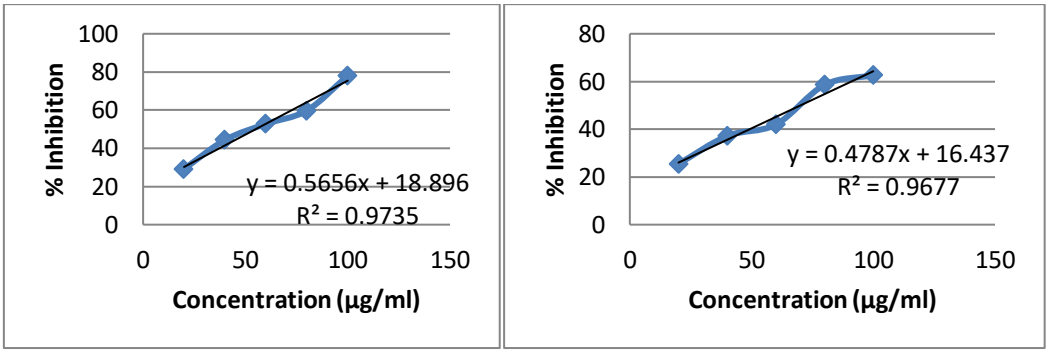
The amount of total flavonoids (mg/gm comparable to rutin)			
Extracts	*Ludwigia adscendens,*	*Launaea pinnatifida*	*Carica papaya*
Absorbance Mean±SD	0.2501±0.004	0.1003±0.002	0.3216±0.003
TPC	81.70	31.76	105.53

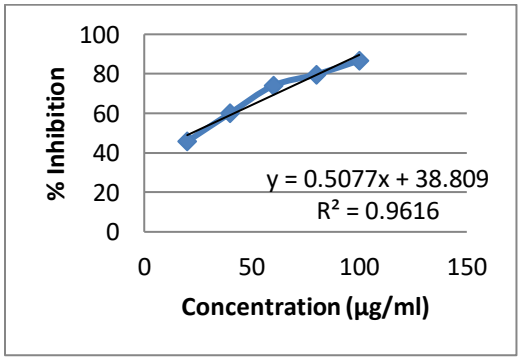
3.5 Anti-oxidant activity

3.5.1 DPPH Assay



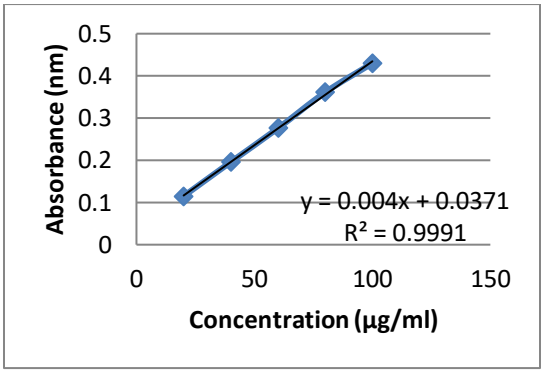
Graph 3: The percentage inhibition vs ascorbic acid concentration is shown in the graph.



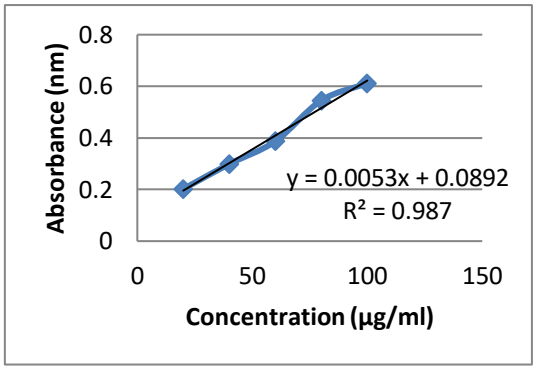


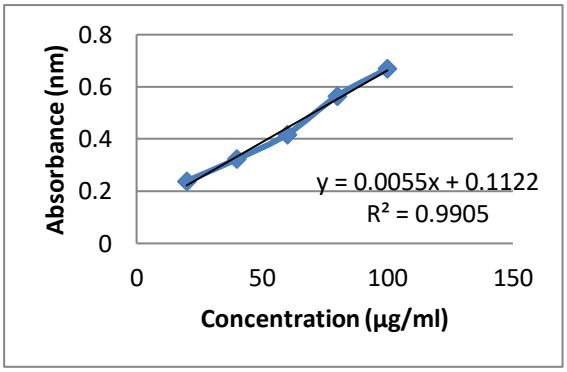
Graph 4: The percentage inhibition versus concentration of hydroalcoholic extracts is shown in the graph of *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya*

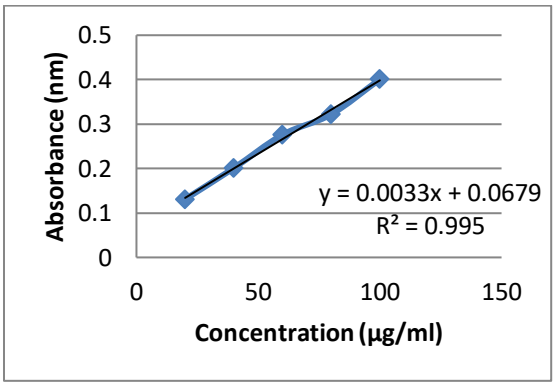
3.5.2 Reducing power scavenging activity



Graph 5: The percentage inhibition vs ascorbic acid concentration is shown in the graph.

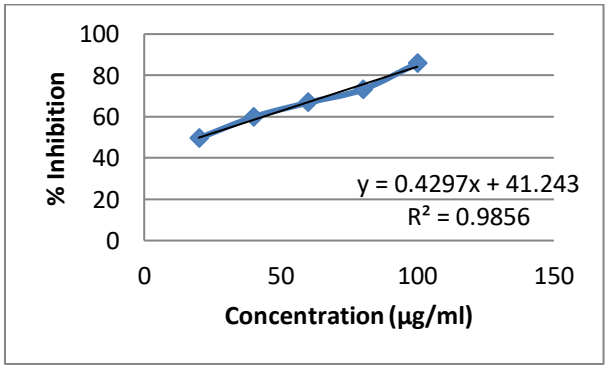




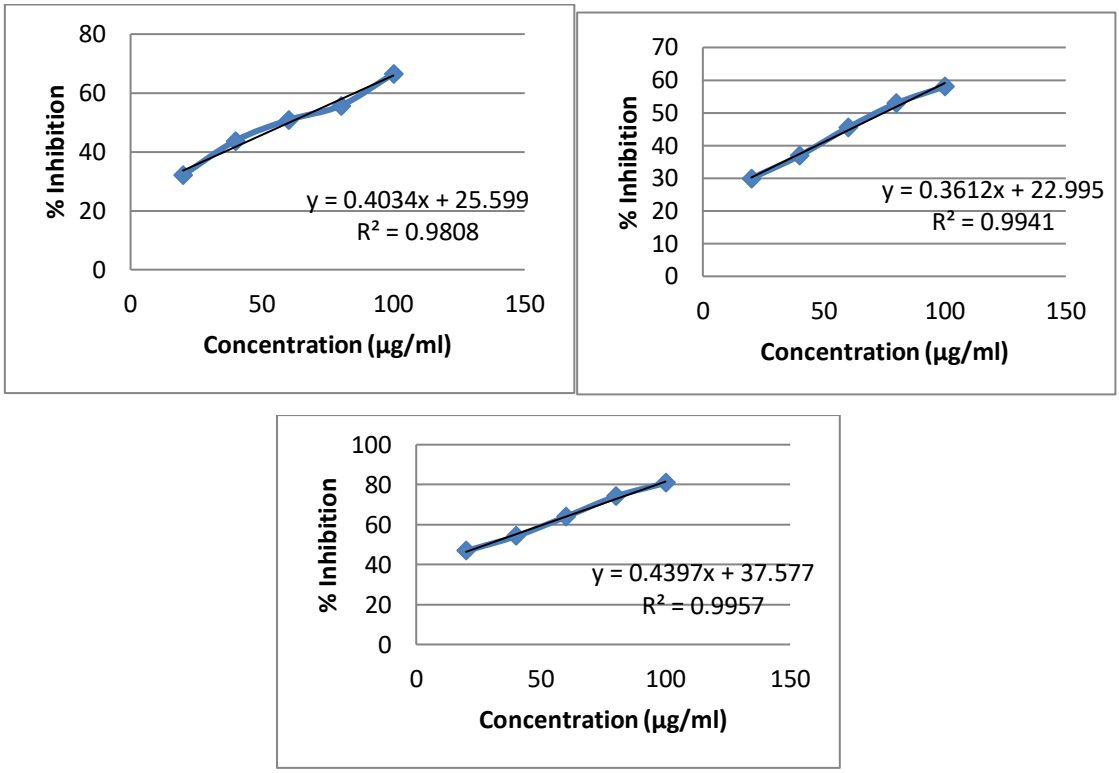


Graph 6: The graph shows the hydroalcoholic extract's absorbance vs concentration for *Ludwigia adscendens, Launaea pinnatifida,* and *Carica papaya.*

3.5.3 Hydrogen peroxide scavenging activity



Graph 7: The percentage inhibition vs ascorbic acid concentration is shown in the graph.



Graph 8: The graph shows the concentration of the hydroalcoholic extract of *Carica papaya, Launaea pinnatifida,* and *Ludwigia adscendens* versus the percentage inhibition.

4. DISCUSSION

The study used *Ludwigia adscendens* Plant (100 gm) and *Launaea pinnatifida* Plant (100 gm) for leaf extraction. The yields of the leaves were 0.85% and 3.12%, respectively. The yields of the leaves in different solvents were 0.481% and 5.82%, respectively. The yields of the leaves in *Carica papaya* Plant were 1.10% and 8.19%, respectively. The total weight of the plants was 100 gm. The study aimed to determine the yields of these plants in different solvents. The Hydro alcoholic extract of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* contains Phytochemical constituents like carbohydrates, alkaloids, flavonoids, terpenoids, steroid, glycosides, protein & amino acid, tannins, phenolic, saponin and compounds by phytochemical investigation with respect to chemical tests. The tpc of the hydroalcoholic excerpts from *Carica papaya, Ludwigia adscendens, and Launaea pinnatifida* was calculated and stated in gallic acid equivalent mg and milligrams for each gram of dry material weight. Phenolic content of Hydro alcoholic extracts showed the content values of 90.86, 38.62 and 124.74 mg/gm respectively. The total flavonoids content of Hydroalcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* were articulated as

percentage of Rutin comparable per mg/gm dry weight of sample. TFC of Hydro alcoholic extracts showed the content values of 81.70, 31.76 and 105.53 mg/gm respectively. Because antioxidants lessen the oxidative damage that ROS cause to biological components, they serve a significant role in shielding our bodies from illness. Current studies have shown that plant-based antioxidants that can scavenge free radicals may be crucial medicinal agents for conditions like diabetes, cancer, cardiovascular illness, neurodegenerative illnesses, ageing, gastrointestinal disorders, arthritis, and the ageing process[34]**.** A variety of in vitro methods, encompassing the DPPH, assay of reducing power along H2O2 assay, were assessed the antioxidant capacity of plant extracts. The antioxidant found *in vitro* of *Carica papaya, Launaea pinnatifida, and Ludwigia adscendens* were assessed in this study. Activity of DPPH radical scavenging Hydro alcoholic excerpts of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* exhibited percent inhibition 78.03, 62.62 and 86.77 % and its IC_{50} value were found to be 55.061, 70.230 and 22.090 µg/ml. As a reference molecule, ascorbic acid demonstrated an IC50 value of 15.569 µg/ml and a proportion of inhibition of 89.51%. Similary, Hydrogen peroxide scavenging assay of Hydro alcoholic Extracts of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* exhibited percent inhibition 66.51, 58.07 and 80.85 % and its IC50 value were found to be 60.570, 74.819 and 28.314 µg/ml. As a reference medication, ascorbic acid showed an inhibition percentage of 85.84 percent and an IC50 value of 20.419 µg/ml.

One important measure of a substance's possible antioxidant action is its reducing capacity. Ascorbic acid and other dietary antioxidants were utilised as a comparison. Reducing power compounds can function as main and secondary antioxidants due to their ability to donate electrons and reduce the oxidised intermediates of lipid peroxidation processes. In contrast to ascorbic acid, the hydroalcoholic extracts demonstrated lowering capacity.

5. CONCLUSION

The study successfully extracted and evaluated the phytochemical and antioxidant properties of *Ludwigia adscendens*, *Launaea pinnatifida*, and *Carica papaya*. The findings verified the existence of bioactive substances that contribute to their powerful antioxidant capacity, such as alkaloids, phenolics, and flavonoids. These findings highlight the medicinal value of these plants and their potential applications in pharmaceutical and nutraceutical industries. Further research is recommended to explore their therapeutic properties and possible commercial utilization.

6. DISPUTE OF INTEREST

A conflict of interest does not exist.

7. ACKNOWLEDGEMENT

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