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Extraction, Phytochemical and Anti-Oxidant Evaluation of Ludwigia Adscendens, Launaea Pinnatifida and Carica Papaya

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ABSTRACT

Aim: This study aims to evaluate the extraction, phytochemical composition, and antioxidant activity of *Ludwigia adscendens, Launaea pinnatifida,* and *Carica papaya* to determine their potential medicinal and nutritional benefits. **Materials and Methods:** Plant extracts were prepared using standard extraction techniques. Phytochemical screening was conducted to identify bioactive compounds such as flavonoids, alkaloids, and phenolics. Antioxidant activity was assessed using DPPH and FRAP assays to measure free radical scavenging potential. **Results:** The phytochemical analysis confirmed the presence of significant bioactive compounds in all three plant species. *Carica papaya* exhibited the highest flavonoid and phenolic content, while *Ludwigia adscendens* and *Launaea pinnatifida* showed moderate levels. Antioxidant assays revealed strong free radical scavenging activity, particularly in *Carica papaya*, indicating its potential as a natural antioxidant source. **Conclusion:** The findings suggest that *Ludwigia adscendens, Launaea pinnatifida*, and *Carica papaya* contain valuable phytochemicals with notable antioxidant properties. These plants hold promise for pharmaceutical and nutraceutical applications, warranting further investigation into their therapeutic potential.

1. INTRODUCTION

Traditionally, plants are viewed as the key source of medicines and play a major part in the health of the overall community. Plant secondary metabolites have long been recognized for their biological impacts on humans. Various plant components, such as bark, leaves, fruits, flowers, and roots, are recognized to have major medicinal qualities due to the presence of a variety of bioactive metabolites. Ornamental plants are often grown for aesthetic purposes in home gardens[1]. Medicinal plants have been widely recognized for their therapeutic potential due to the presence of bioactive compounds with pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and 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,

Journal of Molecular Science
Journal of Molecular Science
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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 contain a diverse array of phytochemicals, including 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 widely distributed in wetland ecosystems of tropical and subtropical regions[7]. It is traditionally used in folk medicine for treating inflammatory disorders, liver ailments, and gastrointestinal complications[8]. The plant is rich in bioactive compounds such as flavonoids, tannins, and polyphenols, which have been reported to exhibit antioxidant and hepatoprotective properties[9]. Studies have suggested that the antioxidant potential of *L. adscendens* is primarily attributed to its ability to scavenge free radicals and modulate oxidative stress pathways. *Launaea pinnatifida*, a member of the Asteraceae family, is a halophytic medicinal plant found in 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 rich source of phytoconstituents such as flavonoids, phenolics, and sterols, which contribute to its antioxidant and hepatoprotective activities[12]. Several studies have demonstrated the free radical scavenging potential of *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 known as papaya is a tropical fruit-bearing plant widely cultivated for its nutritional and medicinal benefits[15]. Various parts of *C. papaya*, including 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 compounds such as alkaloids, flavonoids, tannins, carotenoids, and vitamins, which contribute to its strong antioxidant potential[18, 19]. Studies have reported that papaya extracts exhibit potent free radical scavenging activity, reducing oxidative stress-related complications. Given the increasing interest in natural antioxidants, the present study aims to investigate the extraction, phytochemical composition, and antioxidant evaluation of *Ludwigia adscendens*, *Launaea pinnatifida*, and *Carica papaya*. 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. MATERIAL AND METHOD:

2.1 Plant collection

The medicinal plants *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya* (100 gm) were collected from the local area of Bhopal. After cleaning, plant parts (leaves) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration. Authentication of selected traditional plants - Medicinal plants *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya* were authenticated under Reference nos: 2023071, 2023072 and 2023073 respectively from Govt. College Khimlasa, Sagar, (M.P) on the 26-09-2023 by a plant taxonomist in order to confirm its identity and purity.

2.2 Soxhlet extraction:

Dried and powder of leaves of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using hydroalcoholic solvent system at 40-60^{o}C temperature of the heating mantle for 8-10 hours. After the extraction process, the extracts of samples were filtered and concentrated to dryness. Extracts were collected in air tight container[24, 25]**.** Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

2.2 Qualitative Phytochemical Estimation of Extracts:

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in petroleum ether and hydroalcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya* using standard procedures [26, 27]**.**

2.3 Quantitative Phytochemical estimation-

2.3.1 Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content of plant extracts was determined using the Folin-Ciocalteu Assay. The

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 286-293
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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 mixture was kept in the dark for 90 min at 25°C, after which the absorbance was read at 760 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution (20 to 100µg/ml). The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE) per g of dried sample [28].

2.3.2 Spectrophotometric Quantification of Total Flavonoid Content: -

The flavonoid content was determined using Aluminium chloride method [29]. In a 10 ml test tube, 0.5 ml of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* hydroalcoholic extracts individually, 0.15 ml of $NaNO_2$ (5%) and 0.15 ml of $AlCl_3.6H_2O$ (10%) was mixed. After 5 min, 1 ml of NaOH (4%) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 510 nm. The standard curve for total flavonoid was made using rutin standard solution (20 to 100µg/ml) under the same procedure as earlier described. The total flavonoid was expressed as milligrams of rutin equivalents per g of dried fraction [30].

2.4 Activity (*In-vitro* Anti-oxidant Activity)

2.4.1 DPPH Radical Scavenging Activity

The study involved preparing a 0.1mM solution of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol. A 1 mg/ml methanol solution of extracts/standard was prepared, and different volumes of extracts/standard were taken from stock solution and mixed with 2 ml of DPPH reagent. The absorbance was recorded at 517 nm after 30 minutes of incubation in the dark. A control was prepared by adding 3 ml of 0.1mM DPPH solution and incubating for 30 minutes [31]. The percentage antioxidant activity of the sample/standard was calculated using the formula:

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

2.4.2 Reducing power assay:

The process of preparing standard solutions involves dissolving 3 mg of ascorbic acid in distilled water/solvent, dilutions of which are prepared to give concentrations of 20, 40, 60, 80, and 100 µg/ml. Extracts are prepared by dissolving 1mg of dried extracts in 1ml of methanol, and sample concentrations are prepared. The protocol for reducing power involves mixing aliquots of various concentrations of ascorbic acid and extracts in 1.0 ml of deionized water with 2.5 ml of phosphate buffer and 1 ml of potassium ferricyanide. The mixture is then incubated at 50°C in a water bath for 20 minutes, followed by adding 2.5 ml of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. The absorbance is measured at 700 nm in a UV spectrometer, and a blank is prepared without adding extract[32].

2.4.3 Hydrogen peroxide scavenging activity:

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruch et al.[33]. Aliquot of 0.1 mL of extracts (20–100 µg/mL) was transferred into the eppendorf tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

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

3. RESULTS

3.1 Percentage yield

Table 1 Percentage yield of extracts

S. No.	Plant name	Solvent	Colour of extract	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

Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 286-293
DoI-17.4687/1000-9035.2025.039

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

Table 2: Solubility Determination of Hydro alcoholic extracts

S. No.	Solvents	Result		
		Ludwigia adscendens	*Launaea pinnatifida*	*Carica papaya*
1.	Water	Sparingly Soluble	Partially Soluble	Partially Soluble
2.	Ethanol	Soluble	Partially Soluble	Soluble
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	Soluble	Sparingly Soluble	Soluble

3.3 Qualitative Phytochemical Analysis of different extracts

Table 3 Phytochemical analysis of *Ludwigia adscendens*, *Launaea pinnatifida* and *Carica papaya* extracts

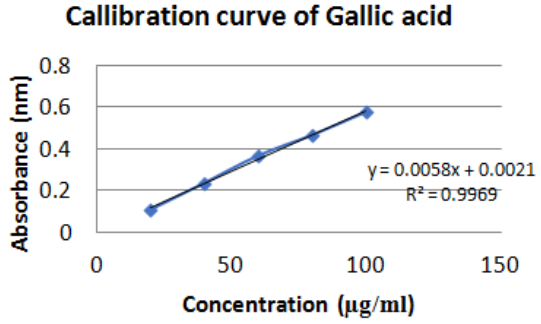
S. No.	Experiment	*Ludwigia adscendens*		*Launaea pinnatifida*		*Carica papaya*	
		Petroleum ether	Hydroalcoholic	Petroleum ether	Hydroalcoholic	Petroleum ether	Hydroalcoholic
Test for Carbohydrates							
1.	Molisch's Test	-	+	-	+	+	+
2.	Fehling's Test	-	+	-	-	-	+
3.	Benedict's Test	+	+	+	+	+	+
4.	Bareford's 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 Phytochemical analysis of Hydro alcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya* -

3.4.1 Total Phenolic Content (TPC) Estimation

Table 4: Standard table for Gallic acid

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: Graph represent standard curve of Gallic acid

Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 286-293
DoI-17.4687/1000-9035.2025.039

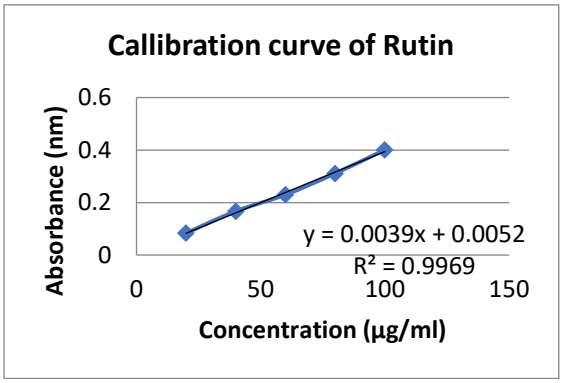
Table 5: Total Phenolic Content in *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* Hydro alcoholic Extract-

Total Phenolic content (mg/gm equivalent to 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 Total Flavonoid Content (TFC) Estimation:

Table 6: Standard table for 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



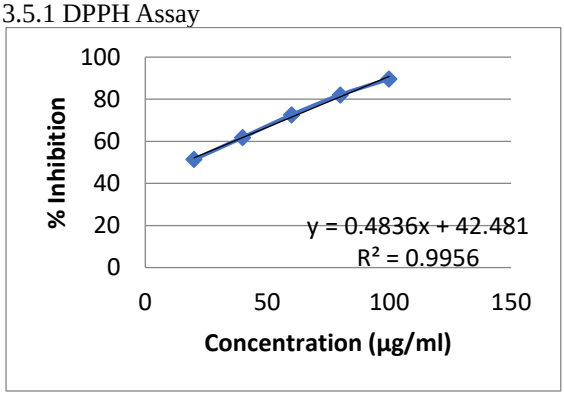
Graph 2: Graph represent standard curve of Rutin

Table 6: Total Flavonoid Content in *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* Hydro alcoholic Extract

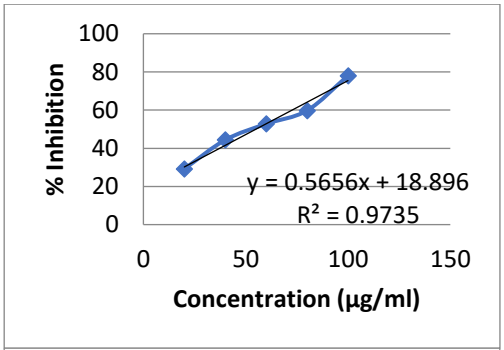
oid content (mg/gm equivalent to Rutin)		
	Ludwigia adscendens,	*Launaea pinnatifida*
	0.2501±0.004	0.1003±0.002
	81.70	31.76

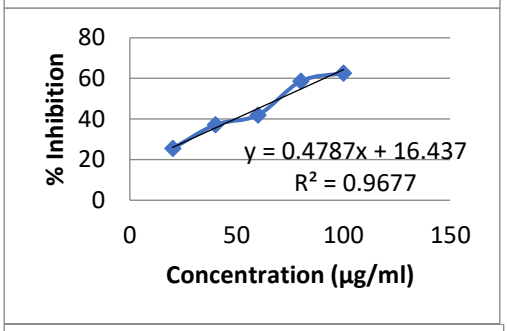
3.5 Anti-oxidant activity

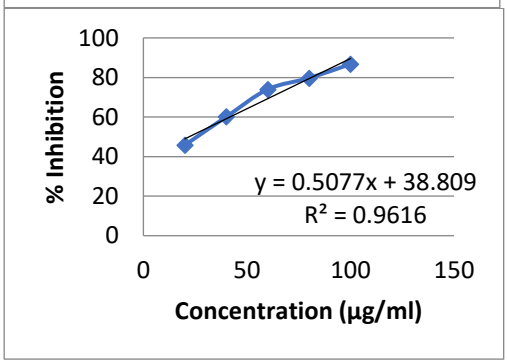
3.5.1 DPPH Assay



Graph 3: Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

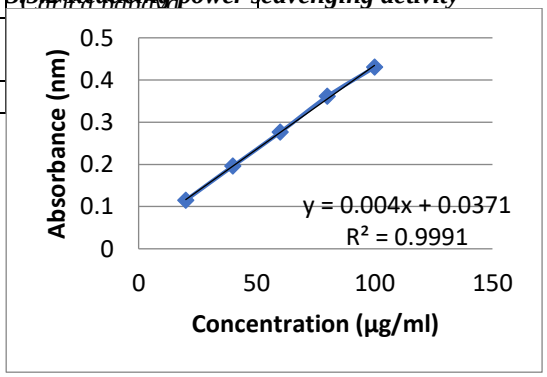






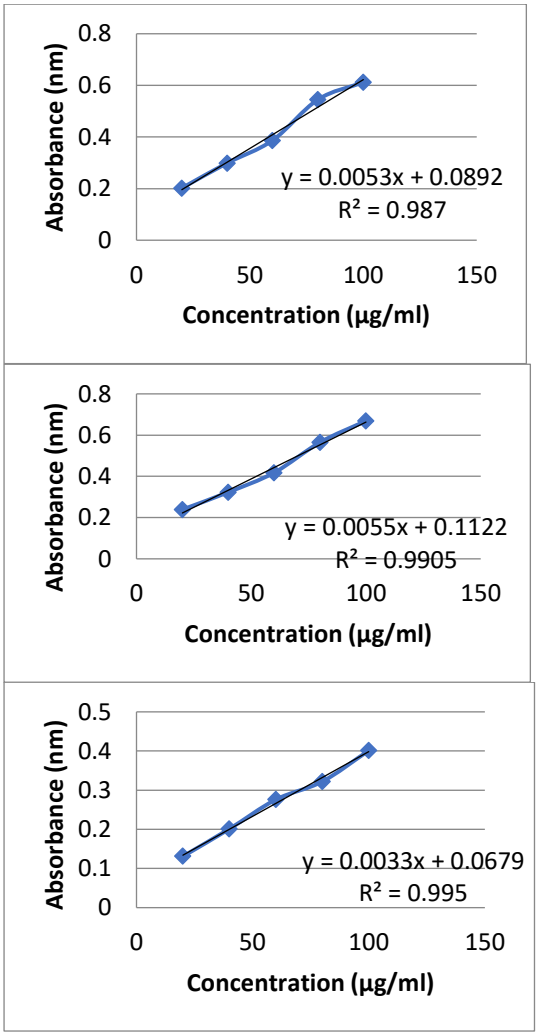
Graph 4: Graph represents the Percentage Inhibition *Vs* Concentration of Hydroalcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida* and *Carica papaya*

3.5.2 Reducing power scavenging activity



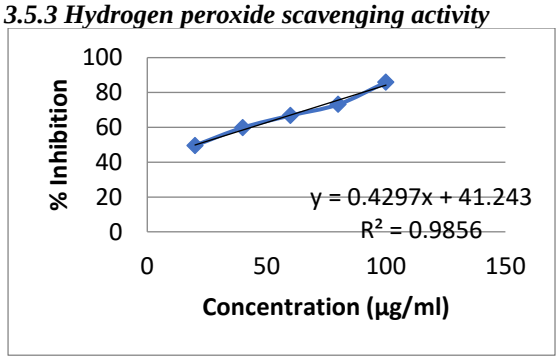
Graph 5 : Graph represents the Absorbance *Vs* Concentration of Ascorbic acid

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 286-293
DoI-17.4687/1000-9035.2025.039

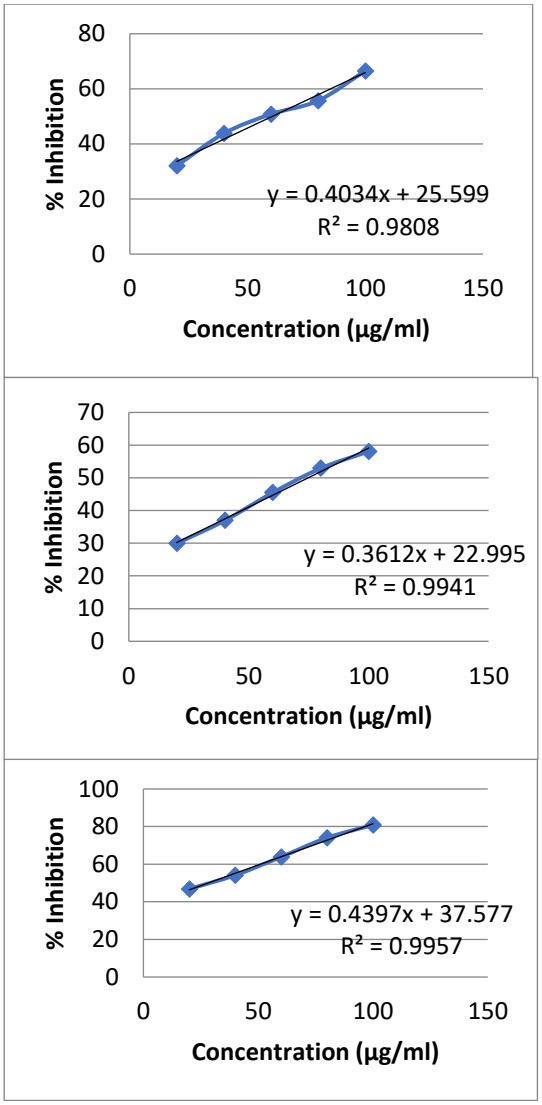


Graph 6: Graph represents the Absorbance *Vs* Concentration of Hydroalcoholic extract of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya*

3.5.3 Hydrogen peroxide scavenging activity



Graph 7: Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid



Graph 8: Graph represents the Percentage Inhibition *Vs* Concentration of Hydroalcoholic extract of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya*

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 determination of the total phenolic content of Hydroalcoholic extracts of *Ludwigia adscendens, Launaea pinnatifida and*

Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 286-293
DoI-17.4687/1000-9035.2025.039

Carica papaya were expressed as mg gallic acid equivalents and per mg/gram dry weight of sample. TPC 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 expressed as percentage of Rutin equivalent per mg/gm dry weight of sample. The total flavonoids content of Hydro alcoholic extracts showed the content values of 81.70, 31.76 and 105.53 mg/gm respectively. Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by Reactive Oxygen Species. Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process[34]. The antioxidant activity of plant extracts was determined by different *in vitro* methods such as the DPPH, Reducing power and Hydrogen peroxide scavenging assay. In this investigation, the in-vitro antioxidant effect of *Ludwigia adscendens, Launaea pinnatifida and Carica papaya* were evaluated. DPPH radical scavenging activity of Hydro alcoholic Extracts 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. Ascorbic acid was used as a reference compound which exhibited percent inhibition 89.61 % and showed IC_{50} value of 15.569 µg/ml. 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. Ascorbic acid was used as a reference compound which exhibited percent inhibition 85.84 % and showed IC_{50} value of 20.419 µg/ml.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. The Hydro alcoholic Extracts showed reducing capacity as compared to Ascorbic acid.

5. CONCLUSION:

The study successfully extracted and evaluated the phytochemical and antioxidant properties of *Ludwigia adscendens*, *Launaea pinnatifida*, and *Carica papaya*. The results confirmed the presence of bioactive compounds, including flavonoids, phenolics, and alkaloids, contributing to their strong antioxidant potential. 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. CONFLICT OF INTEREST:

There is no conflict of interest.

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Journal of Molecular Science
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