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Assessment of Anti-oxidant and Photocatalytic Activity of *Pamburus missionis* Swingle Extracts through GC-MS and ICP-OES Analysis

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***Abstract*:** In this current world of polluted environment, antioxidants play a vital role in mitigating oxidative damage and protecting against harmful effects on human health and the environment by neutralising free radicals. The photocatalytic activity can facilitate the degradation of pollutants and toxic substances. Therefore, the exploration of novel antioxidant and photocatalytic agents from natural resources such as plant extracts is integral for the development of effective therapeutic strategies and tenable solutions to combat these pressing issues. *Pamburus missionis,* a rutaceae member which is native to India and Southeast Asia, has been used in Ayurveda with the name "Kudangal" for different types of digestive, respiratory and skin problems. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, glycosides and steroids. GC-MS analysis unveiled totally 8 compounds from leaf, 5 and 8 from stem and bark extracts. ICP-OES analysis disclosed the different mineral elements such zinc (Zn), iron (Fe), copper (Cu), magnesium (Mg) and so on. In DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, bark extract showed highest antioxidant property (81.99%) followed by leaf(69.34%) and stem (48.42%). The leaf extract exhibited good photocatalytic activity (80.50%) followed by bark(72.88%) and stem, (55.72%). The present study demonstrated the antioxidant and photocatalytic potential of *Pamburus missionis*.

***Keywords*:** Antioxidant, Photocatalytic, GC-MS, ICP-OES, Chemical compounds, Plant extracts

Free radicals (FRs) are generated due to cellular metabolism and exposure to environmental stressors like UV radiation, pollution, smoke. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) shows positive effects on cellular responses and immune function at moderate levels. Excessive concentrations can rise oxidative stress, an adverse process that may lead to chronic diseases like cancer, atherosclerosis, neurodegenerative diseases (Baliyan et al 2022). Antioxidants are the compounds, alleviate oxidative damage by FRs through electron donation, radical scavenging and enzymatic activity. Their assistance in neutralizing FRs is vital for maintaining overall health and well-being. Through endogenous production and dietary intake, humans acquire a range of antioxidants, including both confirmed and putative compounds. Higher plants synthesize a diverse array of secondary metabolites, especially polyphenols and flavonoids represent potent source of antioxidants (Aryal et al 2019). These phytochemicals participate in various biochemical pathways and play a crucial role in combating oxidative stress, thereby protecting against various health issues (Pung Rozar et al 2024). For assessing antioxidant activity, DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay is widely employed and affordable technique. DPPH, stable purple colour free radical reacts with antioxidants in the sample and turns into yellow hue, resulting in decrease in absorbance at 517nm (Ramakrishna and Savithramma 2023). Photocatalytic activity of plant extracts refers to the ability of its various bioactive compounds to harness light energy for breaking down harmful pollutants such as methylene blue, paving the way for innovative environmental remediation strategies (Zhang et al 2019).

The increasing concern over environmental pollution and human health has sparked interest in exploring sustainable and ecofriendly solutions from natural resources. Medicinal plants, in, particular have garnered significant attention due to their potential antioxidant and photocatalytic properties. The integration of both these properties in a single material has significant implications for the development of multifunctional therapeutics and environmental technologies. Medicinal plants have been used for centuries as folk medicine, providing a foundation for exploring their antioxidant activity and photocatalytic potential and are widely available, making them a viable option for large scale applications and they also offer environmentally benign alternative for synthetic materials.

Pamburus missionis, a tropical plant species native to western ghats of India has been used in ancient traditional medicinal system for its benefits. In earlier studies, phytochemical analysis was performed using different solvents and observed the presence of alkaloids, phenols, tannins, flavonoids, steroids, glycosides and coumarins in different proportions in different parts (Yaswanthi et al 2024). However, it's antioxidant and photocatalytic activity remains

largely unexplored. This study aims to investigate these properties of this plant extracts using Gas Chromatography-Mass Spectrometry (GC-MS) and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis. GC-MS will facilitate the identification and quantification of bioactive compounds responsible for antioxidant activity, while ICP-OES will enable the determination of elemental composition and photocatalytic potential. The findings of this research contribute to the understand *Pamburus missionis'*s therapeutic and environmental applications, providing valuable insights into its potential as a sustainable source of antioxidant and photocatalysts.

MATERIAL AND METHODS

Pamburus missionis, belongs to Rutaceae, with only one species and is evergreen tree, 10-15 tall, branched with stout-straight spines (Fig. 1). Leaves are elliptical, dark green, 6-10 cm long. Flowers are white, tetramerous and Fruits, globose berry highly glandular (Fig. 2). The parts were collected from Mamandur forest, beside Balupalle, Karakambadi Rural, Tirupati, Andhra Pradesh *GPS:* 13°46'02.6" N; 79°26'02.5" E. They are thoroughly washed and shade dried about 20 days and ground into fine powders, stored for further study.

Plant extracts preparation: One g of powdered plant materials were dissolved in 20mL of distilled water (DW) and subjected to thermal extraction. The mixtures were heated on a water bath at 60°C for 20 minutes and then allowed to stand overnight at room temperature. Following incubation, the mixtures were filtered to obtain crude extracts.

Methods

DPPH assay: DPPH stock solution was prepared by dissolving 10mg of DPPH in 100mL of Methanol, which yielded a solution mixture with an absorbance of around 1.305 at 517 nm. In the test tubes, 3 mL DPPH workable solutions (1mL of DPPH stock solution + 2mL of methanol) were combined with 100 µL of leaf, stem and bark extracts respectively. As a standard, 3mL of DPPH workable solution often mixed with 100µL of methanol. After 30 min incubation in complete darkness, the absorbance was therefore determined at 517 nm. The percentage of antioxidants was estimated (Ramakrishna and Savithramma 2019, Baliyan et al 2022).

$$\text{Percentage of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where: Ac-Control reaction absorbance; As-Testing specimen absorbance.

Methylene blue dye degradation: Methylene blue (MB) is a commonly used model pollutant for photocatalytic degradation studies. 10mg of MB was combined with 1L of DW. To 100mL of this solution, 10 mg of Plant powders were added and kept under the sunlight. The absorbances were noted at 664nm using UV-VIS spectroscopy after 5 min, 15 min, 30 min and 60 min of incubation. The percentage of dye degradation was calculated by using below formula (Yugandhar et al 2012):

$$\text{Percentage of dye degradation} = ((A_i - A_f)/A_i) \times 100$$

Where A_i = Absorbance initial; A_f = Absorbance final

GC-MS analysis: GC-MS analysis was used to identify and quantify the specific compounds in the plant's extracts. *P. missionis* was subjected to this test to identify the novel compounds that were aiding in antioxidant activity. Methanolic extracts were prepared by soaking 100mg of plant powders in 1mL of methanol for 24 h at room temperature. The mixture was filtered and performed analysis using GC-MS QP2010, SHIMADZU (Konappa et al 2020).

Fig. 1. *Pamburus missionis*

Fig. 2. Leaves and fruit

ICP-OES analysis: ICP-OES analysis is a Spectro analytical technique used to detect and quantify elemental concentrations. To identify elemental composition of Different parts of the *P. missionis*, it was subjected to ICP-OES analysis using Perkin Elmer 7000DV ICP-OES model. 100mg of plant powders were digested with 1ml of 30% of $H_2O_2$and 7mL of 70% $HNO_3$and kept in a muffle furnace for 10 min at 170°C. Then these were filtered and made upto 25mL and performed the analysis (Yugandhar and Savithramma 2017).

RESULTS AND DISCUSSION

The aqueous extracts of *Pamburus missionis* exhibited potent DPPH radical scavenging activity (Table 1). The results demonstrated that the bark had the high scavenging activity (81.99%), followed by the leaf (69.34 %) and the stem showed less activity comparably. Through GC-MS analysis, various bioactive compounds are identified in the leaf, stem and bark with their potential uses (Table 2-4). In leaf, total eight compounds were found. 2- butynoic acid which is a synthon in variety of reactions, including cycloacylation of phenols to flavones & chromones. Ethane,1-chloro-1-fluoro is an effective catalyst and Hydrogen halide scavenger for hydrogen fluoride and hydrogen chloride (NCBI 2024) 1,4-Dimethyl-5-oxabicyclo [2.1.0] pentane is also found in *Chara baltica, Dysphania ambrosioides*. (Tatipamula 2019). Cyclotrisiloxane, hexamethyl exhibit somewhat antibacterial and antioxidant activities (Momin and Thomas 2020). In stem, out of five compounds identified, three were same as in the leaf and the other two namely, 2- Butanone is sweet odour colourless liquid generally present in some foods like banana, cabbage, citrus fruits etc. (Api 2019) and Octane,1-iodo- no sufficient records. From bark, also eight compounds were discovered. 2-butanone, 3-methyl is commonly found to be present in all the three parts. Propiolic acid exhibit potent antioxidant activity due to presence of alkynyl group, allows it to effectively quench radicals and prevent oxidative damage (Kumar et al 2013). 2-chloroethyl methyl sulfoxide, an intermediate, particularly in the production of pesticides, herbicides and fungicides (Singh et al 2015). Arsenous acid, tris (trimethylsilyl) ester is generally a reagent in organic synthesis, like arsenic-containing compounds which may be utilised for treating cancer and infectious diseases (NCBI 2024). From these results, evidence for antioxidant property

Table 1. Antioxidant activity of *P. missionis* extracts with DPPH

Parameter	Control	Leaf	Stem	Bark
Absorbance at 517nm	1.305	0.400	0.673	0.235
% of antioxidant	--------	69.34%	48.42%	81.99%

Table 2. GC-MS of methanolic extract of leaves

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Uses
1.040	2-Butynoic acid	$C_4H_4O_2$	84	1.027	Anti –inflammatory and anticancerous agent, Synthon
1.094	Ethane, 1-chloro-1-fluoro	C_2H_4ClF	82	93.852	Catalyst,
1.144	Ethanol	C_2H_6O	46	1.106	Disinfectant and Antiseptic
1.169	1,4-Dimethyl-5-oxabicyclo [2.1.0] pentane	$C_6H_{10}O$	98	0.079	----------
1.200	Dimethyl sulfide	C_2H_6S	62	2.370	Gas odorant, catalyst, impregnator, food flavoring agent, anti-coking agent.
1.420	N-Nitroso-2-methyl-oxazolidine	$C_4H_8N_2O_2$	116	0.395	Liver Carcinogen
1.644	2-Butanone,3-methyl	$C_5H_{10}O$	86	0.158	Intermediate for production of herbicides and dye precursors
2.997	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222	0.316	Antibacterial and antioxidant, Softening agent in textile.

Table 3. GC-MS of methanolic extract of stem

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Uses
1.091	Ethane, 1-chloro-1-fluoro	C2H4ClF	82	98.973	Catalyst,
1.394	2-Butanone	C4H8O	72	0.391	Cleaning agent
1.636	2-Butanone, 3- methyl-	C5H10O	86	0.195	Intermediate for production of herbicides and dye precursors
1.786	Octane 1-iodo-	C8H17I	240	0.097	----------
2.975	Cyclotrisiloxane, hexamethyl-	C6H18O3Si3	222	0.0195	Antibacterial and antioxidant, Softening agent in textile.

of this plant's parts, especially, Cyclotrisiloxane, hexamethyl in leaves and stem were responsible for their antioxidant nature while, propiolic acid in bark a dominant antioxidant, hence expressed better activity than other parts extracts.

Methylene blue dye degradation is a widely studied process in photocatalysis and environmental remediation. The experiment with plant powders demonstrated the leaf's excellent photocatalytic property (80.5%), followed by the bark and stem (Table 5). Flavonoids, polyphenols and chlorophylls play a vital role in this property, also, elemental composition contribute its part. To unveil the composition of different elements, the powders were analysed using ICP-OES technique. Through this test, total 11 elements and their quantities were determined (Table 6). Among those, mainly zinc, copper and iron are essential for photocatalytic activity. The leaf's photocatalytic efficiency was significantly boosted by its elevated levels of these micronutrients and also due to required amounts of phenols, flavonoids and tannins. Though the stem has good amounts of these elements, it has reduced amounts of secondary metabolites when compared to bark. Therefore, the bark expressed better photocatalytic activity than stem. The increase in demand for sustainable materials with antioxidant and photocatalytic properties had led to renewed interest in medicinal plants because of their easy availability and ecofriendly nature. *Pamburus* is one of such plant, has to be explore more to understand its potentials on various applications. The previous study on secondary metabolites of this plant, enhanced the research

Table 5. Photocatalytic activity through Methylene dye degradation by *Pamburus missionis*

Time	Dye degradation (%)		
	Leaf	Stem	Bark
5 min	41.31	15.89	21.18
15 min	47.46	22.88	29.66
30 min	65.46	49.57	54.87
60 min	80.50	55.72	72.88

Table 4. GC-MS of methanolic extract of bark

Retention time	Name of compound	Molecular formula	Molecular weight	Peak area (%)	Uses
1.007	Argon	Ar	40	1.913	Used in lasers, medical imaging, food packaging etc.
1.032	Propiolic acid	C3H2O2	70	1.177	Antioxidant, antimicrobal, corrosion inhibitor and UV stabilizers
1.075	2-Chloroethyl methyl sulfoxide	C3H7ClOS	126	93.472	Intermediate in the production of antibacterial, antifungal and antiviral agents
1.125	Ethanol	C2H6O	46	0.294	Disinfectant and Antiseptic
1.169	Di-isopropyl ether	C6H14O	102	1.030	Solvent in organic synthesis, pharmaceuticals.
1.377	2,4-Pentanedione, 3-methyl	C6H10O2	114	0.883	Flavor and fragrance, intermediate in organic synthesis, corrosion inhibitor
1.624	2-Butanone,3-methyl	C5H10O	86	0.588	intermediate for production of herbicides and dye precursors
2.055	Arsenous acid, tris(trimethylsilyl) ester	C9H27AsO3Si3	342	0.588	Used in the synthesis of arsenic based pharmaceuticals for treating cancer and infectious diseases.

Table 6. ICP-OES of *Pamburus missionis*

Name of the element	Units	Leaf	Stem	Bark
Nitrogen (N)	%	0.94	2.01	1.2
Phosphorous (P_2O_2)	%	0.1437	0.1028	0.1038
Potassium (K_2O)	%	2.254	1.832	1.009
Calcium (Ca)	%	2.556	2.116	0.7918
Magnesium (Mg)	%	0.4254	0.2613	0.0329
Zinc (Zn)	ppm	17.86	21.89	10.80
Iron (Fe)	ppm	235.3	211.5	132.1
Copper (Cu)	ppm	11.82	45.83	5.105
Manganese (Mn)	ppm	13.43	196.3	35.92
Boron (B)	ppm	55.34	28.45	6.203
Molybdenum (Mo)	ppm	4.200	52.28	3.900

interest to discover its biological activities which led to the present study. Furthermore, research is essential to uncover its capabilities.

CONCLUSION

The comprehensive evaluation of *Pamburus missionis* revealed its remarkable antioxidant and photocatalytic properties, underscoring its potential as a versatile natural resource. The identification of diverse bioactive compounds and essential elements highlights its therapeutic and environmental applications. The findings suggest that *Pamburus missionis* could be a valuable source of natural antioxidants and photocatalysts, warranting further investigation for its potential uses in environmental remediation like wastewater treatment and pollution control, and in medicinal applications as antioxidant supplements, antimicrobial agents etc.

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