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Research

Investigation of Phytochemicals and Antioxidant Properties of *Piper Hymenophyllum* Miq.

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Abstract:
The genus *Piper* stands out as the most extensive within the Piperaceae family, showcasing a diverse array of species that are prevalent across tropical regions. A variety of *Piper* species have been historically utilized for multiple purposes, particularly in medicinal applications, owing to their rich phytochemical compositions. In the commercial market, the pepper of Piperaceae stands out as the most traded spice globally. *Piper* species have been utilized in traditional medicine across the globe to address various health issues, including urological disorders, skin conditions, liver and stomach ailments, wound healing, and to provide antipyretic and anti-inflammatory effects. *Piper hymenophyllum* (Vaal thippili) is an endemic plant native to South India, known for its use by the Kani tribals in the treatment of gastric ailments. Although it has been traditionally utilized, there is a scarcity of documented evidence in the literature concerning its phytochemical properties. This study investigated the pharmacological activities of lesser-known wild piper species, specifically focusing on the leaf, stem, and fruit of *P. hymenophyllum*, thereby enhancing our understanding of its potential therapeutic applications and biochemical properties. The objective of the study was addressed through qualitative preliminary screening and confirmatory tests, which identified the presence of both primary and secondary metabolites. Quantification of total phenolic content revealed the highest concentration in the ethyl acetate leaf extract (247.18 mg GAE/g extract). Similarly, flavonoid content was found to be highest in the ethyl acetate extracts of *P. hymenophyllum* leaf, fruit and stem, ranging from 241 to 304 mg RE/g extract. *In vitro* antioxidant assays (DPPH, ABTS, Superoxide radical scavenging and Phosphomolybdenum) demonstrated that the antioxidant activity was consistently highest in the ethyl acetate extracts of *Piper hymenophyllum* leaf, fruit and stem across all assays. This study provides the detailed evidence of the phytochemical richness and antioxidant potential of *P. hymenophyllum*, scientifically validating its traditional medicinal use and highlighting its promise as a valuable source of bioactive compounds for future pharmacological applications.

Keywords: Phytochemical Screening, Antioxidants, *Piper hymenophyllum*.

1. Introduction

Traditional system of medicine is an important component where 80% of the entire population rely on it. In poor countries, majority of the rural population use these drugs as the first defence in health treatment[1]. Medicinal herbs are more advantageous than modern medicine in treatment of ailments and these also add up some benefits such as treatment efficacy, inexpensive cost and low side effects[2,3]. Numerous medicinal plants have been recognized globally and have been employed for the identification and development of medications

for the beneficial of human health[4,5]. Reactive oxygen species are identified to play an important role in tissue damage, which leads to production of free radicals which causes damage in protein, lipids and DNA leading to chronic disorders such as ageing, atherosclerosis, cancer, cardiovascular disease, diabetes and inflammatory diseases[6]. Synthetic antioxidants such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) were utilized commercially worldwide in food goods and pharmaceuticals which have very dangerous negative effects on human health[7]. Nature has endowed mankind with numerous plant species and phytochemicals which possess variety of biological activity and antioxidant property[8]. Piperaceae is one of the largest medicinal plant families which comprises many valuable natural chemicals. The genus *Piper* contains more than 700 species[9,10]. These plants are typically prevalent in the tropical regions as shrubs, climbing herbs or trees. *Piper* is one of the most often used medicinal plants globally, in traditional system of medicine, the whole plant parts plays a vital role. These species are very much beneficial in treatment of many ailments such as fever, headache, diarrhoea, rheumatism, boils, scabies, respiratory trouble and stomach disorders[11-15]. Bioactive chemicals of *Piper* species have a key role in drug discovery[16]. *P. hymenophyllum* is a wild and underexploited *Piper* species, which is endemic to the Nilgiris Mountains of the Western Ghats. Traditional knowledge from the Kani tribals stresses its usage for gastrointestinal disorders[17]. Despite minimal phytochemical studies on *Piper hymenophyllum*, the literature reported its antibacterial capabilities. The essential oil produced from the leaves of *P. hymenophyllum* was shown to include Phytol as a significant constituent, constituting 21.87% of the oil, along with 24 other recognized chemicals[18]. Investigation of the aerial parts of *P. hymenophyllum* for cholinesterase inhibitory activity and successfully isolated a novel compound, N-(3,5-dimethoxy-4-hydroxycinnamoyl) pyrrole, along with six known compounds, all of which demonstrated acetylcholinesterase (AChE) inhibitory properties, suggesting potential applications for treating Alzheimer's disease[19]. Similarly, identification a novel oxoaporphine alkaloid, (-)-(6aR,7R)-N-acetylrashinsunine, along with eight additional secondary metabolites[20]. In Sri Lanka, a survey had done and found that the *P. hymenophyllum* is an extremely rare wild species[21].The present work intends to evaluate the antioxidant potential of this species, expanding its phytochemical and pharmacological characterisation.

2. MATERIALS AND METHODS

2.1. Plant Sample Collection and Extract Preparation

The Fresh leaves, fruits and stems of *P. hymenophyllum* were collected from Kotagiri, The Nilgiris, Tamil Nadu, India during March 2023 and their taxonomic identity was validated by the Botanical Survey of India, Southern Circle, Coimbatore. The plant materials were washed, shade-dried, and pulverized. Sequential extraction was done using Soxhlet apparatus with solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate, and ethanol. A final aqueous extraction with hot water was done by hot-maceration techniques.

2.2. Qualitative Phytochemical Screening

The leaf, fruit and stem extract of *P. hymenophyllum* were analyzed for the presence of major phytochemicals such as carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, tannins, flavonoids, glycosides, flavanol glycosides, cardiac glycosides, phytosterols, fixed oils & fats and gums & mucilages according to standard methods[22].

2.3. Quantification of Total Phenolic and Total Flavonoid Content

The total phenolic content was assessed using the Folin-Ciocalteu technique[23]. Plant extracts (1 mg/mL) were combined with Folin-Ciocalteu reagent and sodium carbonate solution. After incubation in the dark for 40 minutes, absorbance was measured at 725 nm. The data were represented as gallic acid equivalents. The total flavonoid content was quantified by combining the extracts with sodium nitrite, aluminum chloride and sodium hydroxide. After incubation, absorbance was obtained at 510 nm. Rutin served as the standard, and the results were represented as rutin equivalents[24]. All experiments were performed in triplicates.

2.4. Antioxidant Assays

The antioxidant activity of the extracts was analysed by DPPH radical scavenging activity assay[25]. Various quantities of extracts were combined with 0.1 mM DPPH solution, and

absorbance was recorded at 517 nm after 20 minutes. The IC50 values indicating the concentration required to block 50% of DPPH, were determined. The Total antioxidant activity was also evaluated using ABTS radical decolorization[26]. After ABTS was reacted with potassium persulfate, the resulting solution was diluted and applied to the sample extracts. Absorbance was measured at 734 nm after 30 minutes, and the results were expressed as µM Trolox equivalents. The ability of the extracts to scavenge superoxide radicals was assessed using the riboflavin-light-NBT method[27]. Absorbance was measured at 590 nm after illumination and scavenging activity was estimated. In addition to that the Phosphomolybdenum Assay[28] was carried out by incubating the samples with reagents and absorbance was measured at 695 nm, then the findings were expressed as mg AAE/g extract. Finally the Reducing power was measured by combining extracts with phosphate buffer and potassium ferric cyanide[29]. After incubation, the reaction mixture was centrifuged, and absorbance was measured at 700 nm.

3. RESULTS

3.1. Qualitative Phytochemical Screening of *Piper hymenophyllum* leaf, fruit, and stem:

Phytochemical screening of *P. hymenophyllum* leaf, fruit and stem extracts revealed the presence of both primary metabolites such as carbohydrates, proteins, and amino acids and secondary metabolites like alkaloids, saponins, phenolic compounds, glycosides, flavonoids, cardiac glycosides, phytosterols, fixed oils and fats. High quantities of various secondary metabolites, represented by the "+++" sign, showed their abundance in the plant, whereas a "–" indicates their absence (Table 1). Plant-derived phytochemicals are well known for their physiologically active molecules, which contribute to numerous therapeutic effects, including antioxidant, antibacterial, antifungal and anticancer activity[30,31]. Secondary metabolites, in particular, have been discovered as significant sources of antioxidant and antibacterial capabilities, which function through multiple biological mechanisms[32].

Table 1: Screening of Preliminary Phytochemical components of *P. hymenophyllum*

PHYTOCHEMICALS	LEAF					FRUIT					STEM				
	P.E	CF	E.A	E	A	P.E	CF	E.A	E	A	P.E	CF	E.A	E	A
Carbohydrates	+++	++	+++	+++	++	+++	++	+++	++	++	++	+	+++	++	++
Proteins	+++	++	+++	+++	+++	+++	+++	++	+++	++	+	+	++	+	+
Amino acids	+++	++	+++	+++	++	+++	+	++	++	++	++	++	++	++	++
Alkaloids	+	++	+	+	++	+	-	-	-	++	++	-	-	++	-
Saponins	+++	++	++	+++	++	+++	+	+	++	++	+	+	+	+	++
Phenolic compounds	++	++	++	++	+	++	+++	++	+++	++	++	++	++	+++	++
Glycosides	+	++	++	+++	++	+	+	+	+	++	+	-	+	+	+
Flavonoids	++	++	++	++	++	++	++	+	++	++	+	++	+	+	++
Cardiac glycosides	+++	++	+++	++	++	+++	++	++	++	++	+	+	++	+	++
Phytosterols	+	+++	++	+++	+	-	+	-	-	+	++	+	-	++	+
Fixed oils and fats	+	+++	++	+	+	+++	++	+	++	++	++	+	+	+	++

leaf, fruit and stem extracts were measured using Folin- Ciocalteu reagent, with gallic acid as the standard ($R^2 = 0.994$). The phenolic content was expressed as mg GAE/g extract, the ethyl acetate extracts of leaf, ethanol extract of fruit and stem showed the highest phenolic content of 247.18, 215.38, and 210.48 mg GAE/g extract, respectively (Fig 1).

Fig.1: Total Phenolic content of *P. hymenophyllum:*

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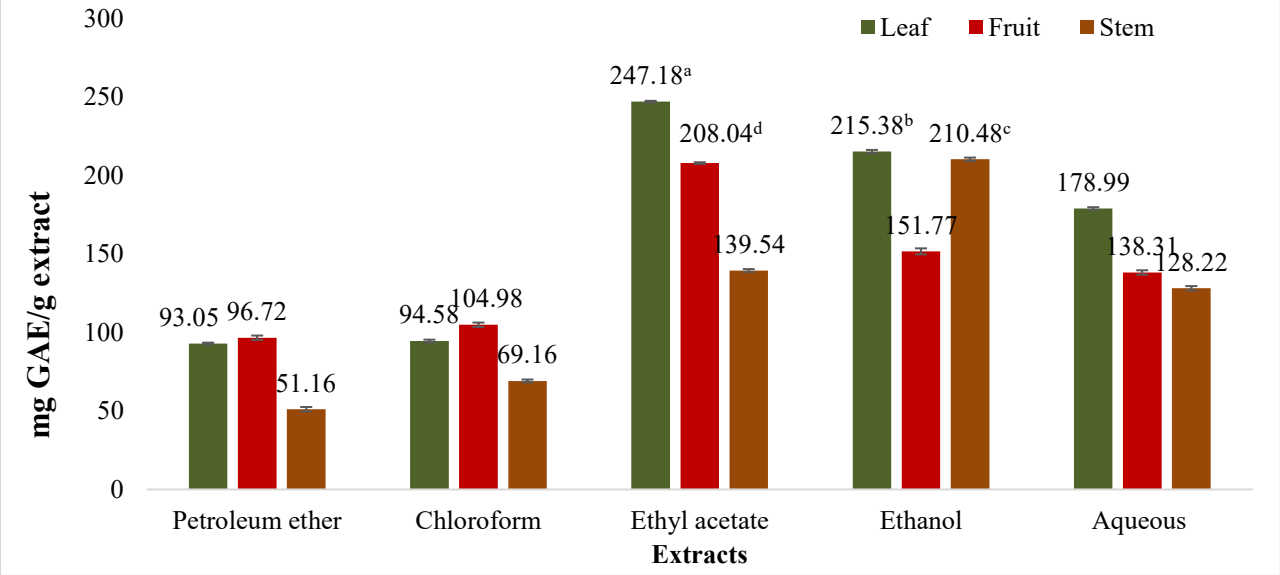
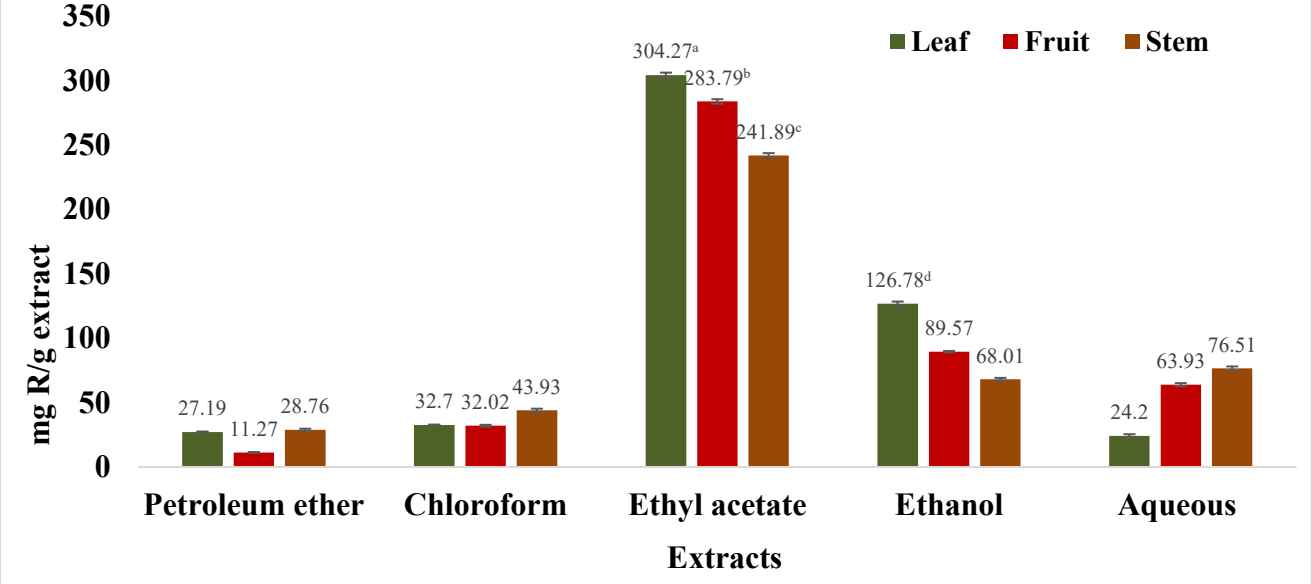


Values are mean of triplicate determination (n=3), GAE- Gallic Acid Equivalent
Statistically significant at $p < 0.05$ where $^{a} > ^{b} > ^{c} > ^{d}$

3.3.2. Quantification of Total Flavonoid content:

The Flavonoids are important secondary metabolites with strong anti-oxidants and protective roles, and their variation among extracts indicates the influence of solvent polarity and the plant- specific distribution. The total flavonoid content was highest in leaf, fruit and stem ethyl acetate extracts ranging from 241 to 304 mg RE/g extract (Fig 2).

Fig.2: Total Flavonoid content of *P. hymenophyllum*:



Values are mean of triplicate determination (n=3), R- Rutin Equivalent
Statistically significant at $p < 0.05$ where $^{a} > ^{b} > ^{c} > ^{d}$

3.4. Antioxidant assays of *P. hymenophyllum* leaf, fruit and stem:

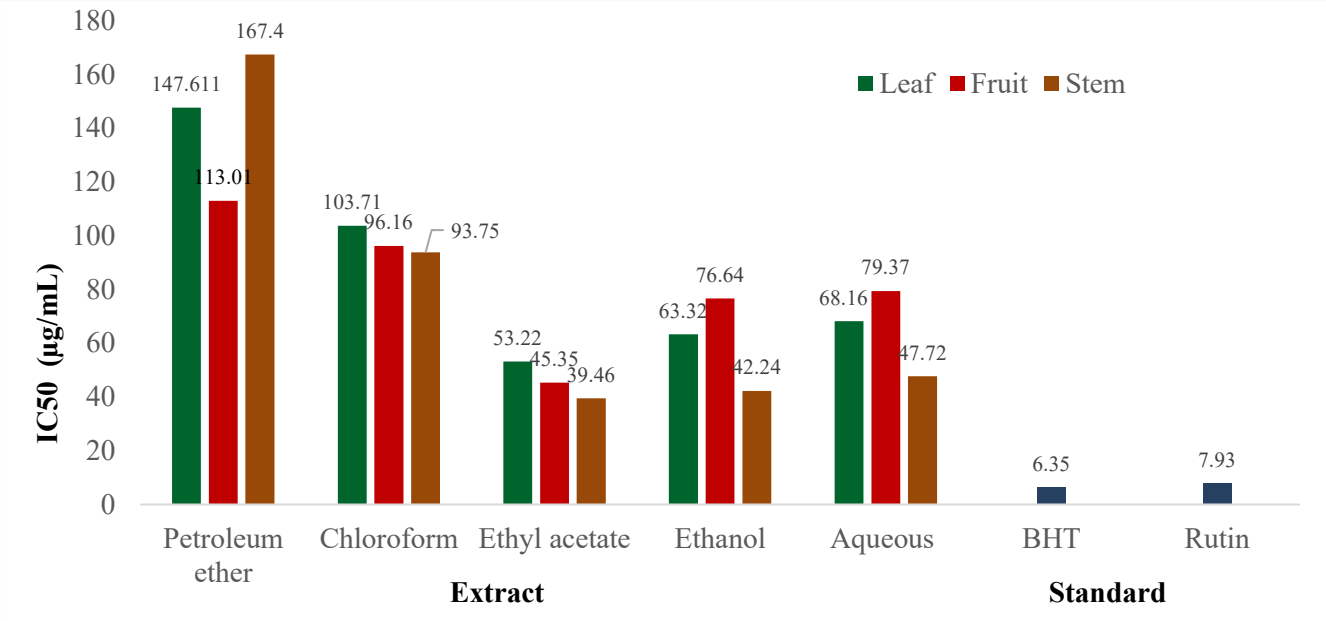
The antioxidants play a crucial role in neutralizing free radicals, protecting cellular components from oxidative damage and thereby contributing to the therapeutic potentials of the plants.

3.4.1. DPPH scavenging activity:

The DPPH radical scavenging activity (IC50) of *P. hymenophyllum* leaf, fruit and stem extracts was examined, representing the concentration necessary to neutralize 50% of the DPPH free radical within 30 minutes. Ethyl acetate extracts demonstrated the greatest antioxidant activity, with IC50 values of 53.22, 45.35, and 39.46 µg/mL for leaf, fruit and stem, followed by ethanolic extracts showed highest efficacy, but petroleum ether extracts displayed much lesser antioxidant activities than the other extracts. Positive controls, Rutin (IC50: 7.93 µg/mL) and BHT (IC50:

6.35 µg/mL), emphasized the substantial antioxidant capability of the *P. hymenophyllum* extracts. Results are shown in Figure 3.

Fig.3: DPPH scavenging activity of *P. hymenophyllum:*



3.4.2. ABTS scavenging activity:

The ABTS scavenging activity of *P. hymenophyllum* leaf, fruit and stem extracts is illustrated in Table 2. Ethyl acetate extracts displayed the highest activity, with values of 69,687.5, 55,555.6, and 66,076.4 µM TE/g extract, respectively, outperforming other extracts. Standards rutin and BHT showed greater activity at 94,166.7 and 95,347.2 µM TE/g, respectively. These data emphasize the great total antioxidant potential of carotenoids, phenolics, and other antioxidants in the extracts.

3.4.3. Superoxide radical scavenging activity

The superoxide radical scavenging ability of *P. hymenophyllum* leaf, fruit and stem extracts was tested by NBT reduction at 560 nm, with decreased absorbance suggesting radical quenching. Ethyl acetate extracts demonstrated the highest inhibition rates of 72.52% (leaf), 70.11% (fruit) and 66.91% (stem) exhibiting considerable activity equivalent to the reference standards BHA and BHT (Table 2).

3.4.4. Phosphomolybdenum assay

The phosphomolybdenum assay examined the total antioxidant capacity (TAA) of *P. hymenophyllum* leaf, fruit and stem extracts (Table 2). Ethyl acetate extracts demonstrated the highest lowering capacity, with 330.71, 292.76, and 161.53 mg AAE/g extract for leaf, fruit and stem, respectively. This approach, based on Mo (VI) reduction to Mo (V), indicates the significant antioxidant capacity likely related to the extract's phenolic and flavonoid composition.

Table 2. Anti- oxidant assays of *P. hymenophyllum* (Leaf, Fruit and Stem)

Samples	Extracts	ABTS Assay mM TE/g (extract)	Superoxide Radical Scavenging Assay (% of Inhibition)	Phosphomolebdenum Assay mg AAE/g (extract)
Leaf	Petroleum Ether	34131.9 ± 677.75	43.42±1.26	159.01±1.3
	Chloroform	40937.5 ± 813.56	51.14± 0.64	172.64±0.62 [d]
	Ethyl- acetate	69687.5 ±1159.95 [c]	72.52± 1.04 [b]	330.71±0.96[a]
	Ethanol	61597.2 ±1294.07	66.85± 0.14 [d]	197.79±1.25[c]
	Aqueous	47083.3 ±721.68	65.74±0.15 [d]	171.17±0.96 [d]
Fruit	Petroleum Ether	30451.4 ± 1027.6	46.14± 1.66	124±0.96
	Chloroform	38784.7 ± 1489	53.65±0.26	127.14±1.58
	Ethyl- acetate	55555.6 ±1517.8	70.11±0.25 [c]	292.76±1.66[b]

	Ethanol	44166.7 ±1637.1		61.91±1.71		144.34±1.25
	Aqueous	40173.6 ± 1421.6		56.8±0.54		123.75±1.45
Stem	Petroleum Ether	56527.8 ± 945.1		55.95±1.76		161.32±1.88
	Chloroform	61423.6 ± 394.3		55.67±0.2		131.34±0.72
	Ethyl- acetate	66076.4 ± 573.7[d]		66.91±1.17 [d]		153.98±1.58
	Ethanol	65833.3 ± 275.5[d]		62.68±1.02		161.53±0.96
	Aqueous	62569.4 ± 781.8		57.39±1.27		128.82±1.92
Standard		**Rutin**	94166.7 ± 416.6 [b]	**BHA**	84.7±0.25 [a]	-
		BHT	95347.2 ± 636.4 [a]	**BHT**	84.2±0.1 [a]	-

Values are mean of triplicate determination, AAE - Ascorbic Acid Equivalents, TE – Trolax,
BHA- Butylated Hydroxy Anisole, BHT- Butylated Hydroxy Toluene.
Statistically significant at $p < 0.05$ where $^a > ^b > ^c > ^d$ in each column.

4. Discussion:

The above studies underscore the rich phytochemical composition and strong antioxidant potential of *P. hymenophyllum.* Preliminary phytochemical screening of *P. betle* leaf extracts revealed that the petroleum ether extract contained alkaloids and phytosterols, while the ethanolic and aqueous extracts tested positive for carbohydrates, proteins, phytosterols, saponins, flavonoids, alkaloids, volatile oils, tannins, and phenols[33], where in *P. hymenophyllum,* the most of the phytocomponents were present in all the tested extracts except alkaloids and phytosterols. In comparison, *Piper betle* showed a total phenolic content of 51.53 mg GAE/g in methanolic extracts, which exhibited a strong correlation with antioxidant activity[34]. However, *P. hymenophyllum* demonstrated a superior phytochemical profile. For instance, the ethyl acetate extract of *P. longum* fruit contained only 17.27 mg QE/g, which is significantly lower than the corresponding value in *P. hymenophyllum*. Additionally, the methanol fruit extract of *P. longum* recorded a DPPH IC_{50} value of 173 µg/mL, indicating that *P. hymenophyllum* extracts are over three times more potent in antioxidant activities[35]. A similar trend was observed in ABTS assays, where *P. longum* exhibited an IC_{50} of 104 µg/mL and an antioxidant capacity of approximately 1,700 µM TE/g—nearly 40-fold lower than that of *P. hymenophyllum*[36]. Moreover, methanol leaf extracts of other *Piper* species, such as *P. guineense*, *P. nigrum*, and *P. umbellatum*, showed 47–52 % superoxide scavenging activity at a concentration of 8 mg/mL. These values were significantly outperformed by *P. hymenophyllum* extracts[37]. Although *P. schmidtii* fruit extracts reported a relatively high total antioxidant capacity of 135.67 mg AAE/g, this value still falls short when compared to the TAC observed in the ethyl acetate extracts of *P. hymenophyllum* [38].

These comparisons underscore the superior phytochemical richness and antioxidant potential of *P. hymenophyllum*. Its broad spectrum of bioactive compounds and high radical scavenging ability suggest that this species holds promise for future phyto-pharmaceutical development and functional food applications.

5. Conclusion:

In conclusion, the ethyl acetate extracts of *Piper hymenophyllum* leaf, fruit and stem revealed strong antioxidant activity across different assays, including DPPH, ABTS, phosphomolybdenum, and superoxide radical scavenging. This great antioxidant potential is attributed to the presence of bioactive substances such as phenolics and flavonoids. Despite its promising bioactivity, *P. hymenophyllum* remains an underexploited species with tremendous medicinal potentials. Ethno botanical data supports its traditional usage in treating cold and gastro-intestinal illnesses, underlining its value in natural treatment. Further investigations are necessary to identify and describe the active molecules responsible for its antioxidant and therapeutic properties. Additionally, in-depth *in-vitro* and *in-vivo* investigations are required to understand the mechanisms of action of these drugs. Such research could establish *P. hymenophyllum* as a valuable and cost-effective source of natural antioxidants, with potential uses in the creation of medicinal agents for human health and well-being.

REFERENCES

1. Goleniowski ME, Bongiovanni GA, Bongiovanni L, Palacio CO, Cantero JJ. Medicinal plants from the Sierra de Comechingones, Argentina. Journal of Ethnopharmacology, 2006; 107: 324–341.
2. Govaerts R. How many species of seed plants are there?. Taxon, 2000; 50: 1085–1090.

3. Schippmann U, Leaman DJ, Cunningham AB. Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues in Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Satellite Event on the Occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture, FAO, Rome. 2002.
4. Abbasi AM, Khan MA, Khan N, Shah MH. Ethnobotanical survey of medicinally important wild edible fruits species used by tribal communities of Lesser Himalayas- Pakistan. J.Ethnopharmacol, 2013; 148: 528–536.
5. Bieski IGC, Leonti M, Arnason JT, Ferrier J, Rapinski M, Violante IMP, Balogun SO, Pereira JFCA, Figueiredo RDCF, Lopes CRAS, Da DR, Pacini A, Albuquerque UP, Martins DT. Ethnobotanical study of medicinal plants by population of Valley of Juruena Region, Legal Amazon, Mato Grosso. Brazil. J. Ethnopharmacol, 2015; 173: 383–423.
6. Islam S, Nasrin S, Khan MA, Hossain ASM, Islam F, Khandokhar P, Mollah MNH, Rashid M, Sadik G, Rahman MAA, Alam HKA. Evaluation of antioxidant and anticancer properties of the seed extracts of *Syzygium fruticosum* Roxb growing in Rajshahi, Bangladesh. BMC Complement. Altern. Med, 2013; 13: 142.
7. Li L, Ham H, Sung J, Kim Y, Lee HJJ. Antioxidant activities of methanolic extracts from four different rose cultivars. J. Food Nutr Res, 2013; 2: 69–73.
8. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi S, Lel RD. Free radicals and antioxidants in human health: current status and future prospects. J. Assoc. Physicians India, 2004; 52: 794–804.
9. Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen CE. Phytochemistry of the genus *Piper*. Phytochemistry, 1997; 46: 597–673.
10. Nascimento JCD, Paula VFD, David JM, David JP. Occurrence, biological activities and 13C NMR data of amides from *Piper* (Piperaceae). Química Nova, 2012; 35: 2288–2311.
11. Tsai IL, Lee FP, Wu CC, Duh CY, Ishikawa T, Chen JJ, Chen YC, Seki H, Chen IS. New cytotoxic cyclobutanoid amides, a new furanoid lignan and anti-platelet aggregation constituents from *Piper arborescens*. PlantaMedica, 2005; 71: 535–542.
12. Chakraborty D, Shah B. Antimicrobial, anti-oxidative and anti-hemolytic activity of *Piper betel* leaf extracts. International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 3: 192–199.
13. Sharkar P, Rahman MM, Haque GZ, Nayeem MA, Hossen MM, Azad AK. Ethnomedicinal importance of the plants in villages in Kushtia Sador and Mirpur Upozila, Bangladesh. Journal of Herbs Spices & Medicinal Plants, 2013. 19: 401–417.
14. Umoh I, Oyebadejo S, Bassey E, Udoh N. Histomorphological study of the effect of chronic consumption of *Abelmoschus esculentus* and *Piper guineense* on the gastric mucosa of Albino wistar rats. International Journal of Pharmaceutical Research and Allied Sciences, 2013; 2: 31–37.
15. Aziz DM, Hama JR, Alam SM. Synthesising a novel derivatives of piperine from black pepper (*Piper nigrum* L.). Journal of Food Measurement and Characterization, 2015; 3: 324–331.
16. Barh D, Barve N, Gupta K, Chandra S, Jain N, Tiwari S, Leon-Sicairos N, Canizalez- Roman A, Rodrigues A, Hassan SS. Exoproteome and secretome derived broad spectrumnovel drug and vaccine candidates in *Vibrio cholerae* targeted by *Piper betel* derived compounds. PLoS One, 2013; 8: 52773.
17. Balasubramaniam V. Survey and documentation of commercially sold medicinal plants in local markets of Velliangiri Hills (Poondi), Coimbatore District, Tamil Nadu, India. International Journal of Recent Advances in Multidisciplinary Research, 2015; 2(12):1047-1055
18. Dai DN, Huong LT, Van Le N, Dung, VT, Son NT. Essential oils of *Piper pendulispicum* C. DC. and *Piper hymenophyllum* Miq.: Chemical composition and antimicrobial activity. Journal of Essential Oil-Bearing Plants, 2023; 26(2): 403–410.
19. Hoang V D, Nguyen PH, Tran MH, Huynh NT, Nguyen HT, Min BS, To DC. Identification of anti-inflammatory constituents from Vietnamese *Piper hymenophyllum*. Revista Brasileira de Farmacognosia, 2020; 30(2): 312–316.
20. Dung HV, Cuong TD, Chinh NM, Quyen D, Byeon JS, Kim JA, Min BS. Cholinesterase

inhibitors from the aerial part of *Piper hymenophyllum*. Bulletin of the Korean Chemical Society, 2014; 35(2): 655–658

21. Edirisinghe RE. Observations on *Piper hymenophyllum* Miq.: A rare wild *Piper* species in Sri Lanka. Ceylon Journal of Science (Biological Sciences), 2009; 38(1): 23-26
22. Raaman N. Phytochemical techniques. New India Publishing Agency. Jai Bharat Printing Press. New Delhi, 2006 ;19-22.
23. Siddhuraju P. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT-Food Science and Technology, 2007; 40(6): 982-990.
24. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry, 1999; 64(4): 555-559.
25. Blois MS.Antioxidant determinations by the use of a stable free radical. Nature, 1958 ;181:1199–1200.
26. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine, 1999; 26(9-10): 1231-1237.
27. Fridovich I. Superoxide dismutases: defence against endogenous superoxide radical. In Ciba Foundation Symposium 65-Oxygen Free Radicals and Tissue Damage, Chichester, 1979; 65: 77-93.
28. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin. E. Analytical biochemistry, 1999; 269(2): 337-341.
29. Hseu YC, Chang WH, Chen CS, Liao JW, Huang CJ, Lu FJ, Yang HL. Antioxidant activities of *Toona Sinensis* leaves extracts using different antioxidant models. Food and chemical toxicology, 2008; 46(1): 105-114.
30. Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT Food Sci. Technol, 2004; 37: 717–721.
31. Siddhuraju P. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT-Food Science and Technology, 2007; 40(6): 982-990.
32. Chen Y, Xie MY, Nie SP, Li C, Wang YX. Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of Ganodermaatrum. Food Chem, 2008; 107: 231–241.
33. Saini SAPNA, Dhiman ANJU, Nanda SANJU. Pharmacognostical and phytochemical studies of *Piper betle* Linn. leaf. Int J Pharm Pharm Sci, 2016; 8(5) : 222-226.
34. Poudel M, Koirala N, Mehta RK, Lamsal A. Estimation of Phytochemical Constituents and Evaluation of Antioxidant Potency of *Piper betle* Leaves. Nepal Journal of Biotechnology, 2024; 12(1): 48-57.
35. Krishna MS, Joy B, Sundaresan A. Effect on oxidative stress, glucose uptake level and lipid droplet content by Apigenin 7, 4'-dimethyl ether isolated from *Piper longum* L. Journal of Food Science and Technology, 2015; 52: 3561-3570.
36. Biswas P, Pandey DK, Shekhawat MS, Dey A, Malik T. Tissue-specific variations of piperine in ten populations of *Piper longum* L.: bioactivities and toxicological profile. Scientific Reports, 2024; 14(1) : 5062.
37. Agbor GA, Vinson JA, Oben JE, Ngogang JY. *In vitro* antioxidant activity of three *Piper* species. Journal of Herbal Pharmacotherapy, 2008; 7(2): 49-64.
38. Pradheeba M, Pugalenthi M, Deepa MA, Kumar SV, Vasukipridharshini G. Evaluation of Phytochemical Profile and *In Vitro* Antioxidant, Anti-bacterial, and Anti-inflammatory activity of *Piper schmidtii* Hook. fil. A Wild Edible Fruit, 2022; 9(1): 191-197.

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