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Qualitative Phytochemical, Secondary Metabolites and Antioxidant Activity of *Piper peepuloides* Roxb. and *Piper hymenophyllum* Miq. Leaves

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Abstract

Piper peepuloides and Piper hymenophyllum a well-known medicinal plant of Piperaceae family is used by many tribal groups to treat inflammation and diabetics in India. In the study, different extracts such as petroleum ether, chloroform, ethyl acetate, ethanol and aqueous of *Piper peepuloides and Piper hymenophyllum* leaf are evaluated for its qualitative phytochemical screening, secondary metabolites and antioxidant activity. Among various extracts, *Piper peepuloides* leaf ethanol extracts showed the maximum amount of phenolics (336.84 mg GAE/g extract), tannin (331.52 mg GAE/g extract) and (176.18 mg RE/g extract) content. It also revealed the presence of highest antioxidant property by estimating DPPH% (IC_{50}: 24.33 µg/mL), ABTS (68645.8 µM TE/g extract), Superoxide (83.69%) radical scavenging activity and Phosphomolybdenum (21.08 mg AAE/g). *Piper peepuloides* leaf extracts have a tremendous amount of antioxidant potential, making them a good source of natural antioxidant supplements for food to protect against oxidative stress-related diseases.

***Key words*:** Antioxidant, *Piper peepuloides*, *Piper hymenophyllum*

As the primary sources of naturally occurring bioactive chemicals, medicinal plants have widespread acclaim across several conventional medical systems for their wide range of pharmacological applications with little adverse effects on living organisms [1-2]. The search for new pharmaceuticals has fueled decades of study into medicinal herbs and other natural remedies [3]. The bioactive chemicals are crucial in the process of developing novel pharmaceuticals. Estimates put the percentage of pharmaceuticals with a natural origin at around 60% [4]. It has been claimed that medications derived from plants can effectively treat a wide range of infectious illnesses, including skin conditions, TB, diabetes, jaundice, hypertension, mental disorders, cancer, and AIDS [5].

Antioxidants are bioactive chemicals found in many therapeutic plants; they include tannins, sulfur-containing compounds, alkaloids, flavonoids, phenolic compounds, vitamins, and more. Antioxidants are crucial for the metabolism of the body and the prevention of several deadly illnesses [6]. Due to their antioxidant qualities, herbal phenolic compounds have been increasingly popular among both scientists and consumers in recent years [7]. There are antioxidants in every component of a plant, but those that have culinary or therapeutic purposes are especially rich in vitamins A, E, and C as well as phenolic compounds including tannins, lignin, stilbenes, phenolic acids, and flavonoids. Due to their scavenging capabilities, antioxidants derived from natural sources, particularly fragrant spice plants, are currently the subject of intense research. Almost every disease is accompanied by some kind of inflammation and pain. The majority of disability and mortality are caused by common inflammatory disorders, including rheumatoid arthritis, asthma, colitis, and hepatitis [8]. Inflammation has recently been linked to cancer, heart disease, and neurological disorders [9]. Medications that are anti-inflammatory and analgesic alleviate moderate to mild discomfort. The analgesic and anti-inflammatory actions of these drugs are due to their inhibition of prostaglandins, which are chemicals that contribute to the development of pain and inflammation and are also involved in the control of body temperature. When pain and inflammation are treated with larger doses for longer periods of time, toxicity and adverse effects are common outcomes of the widespread use of anti-inflammatory and analgesic medications [10].

One of the most extensive families of medicinal plants is the Piperaceae. Worldwide, there are at least 3,000 species belonging to six different genera. The tropics and subtropics are common habitats for these species [11-12]. The majority of the Piperaceae family includes of terrestrial and epiphyte plants, shrubs, vines, and trees. There are about 2000 species of Piper worldwide [13]. Approximately 51 cultivars have been documented from the subtropical and tropical parts of India, with the three most famous species being *P. betle*, *P. longum* and *P. nigrum* [14]. Wild edible pepper cultivars belonging to the Piper sp. family, such as *Piper peepuloides* and *Piper hymenophyllum*, are known as kattuthipili and kattukurumilagu,

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respectively. Hence the main objective of the present investigation is to evaluate the qualitative phytochemical screening, secondary metabolites and antioxidant, properties of the *P. peepuloides* and *P. hymenophyllum*.

MATERIALS AND METHODS

Collection and identification of plant material

The fresh plant materials were collected during the month of October 2023 from Coonoor and Kotagiris, The Nilgiris district, Tamil Nadu, India. The taxonomic identity of the plants was confirmed from the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2023/Tech-836 & 837). The leaf materials were washed under running tap water to remove the surface pollutants. The separated leaves were air dried under shade. The dried samples were powdered and used for further studies.

Extraction of plant material

The powdered leaf materials were packed in small thimbles separately and extracted successively with different solvents such as petroleum ether, chloroform, ethyl acetate and methanol in the increasing order of polarity using Soxhlet apparatus. Each time before extracting with the next solvent, the thimble was air dried. Finally, the sample was macerated using hot water with constant stirring for 24 h and the water extract was filtered. The different solvent extracts were concentrated by rotary vacuum evaporator (Yamato RE300, Japan) and then air dried. The dried extract obtained with each solvent was weighed. The percentage yield was calculated in terms of air-dried weight of extract from each solvent. The stock solution of the extract obtained was prepared (1 mg/mL of respective organic solvents) and used for further analysis.

Extract recovery percentage

The amount of crude extract recovered after successive extraction was weighed and the percentage yield was calculated by the following formula:

$$\text{Extract recovery percent} = \frac{\text{Amount of extract recovered (g)}}{\text{Amount of leaf sample (g)}} \times 100$$

Qualitative phytochemical screening

The leaf powder of *P. peepuloides and P. hymenophyllum* were analyzed for the presence of major phytochemicals such as Carbohydrates, Proteins, Amino acids, Alkaloids, Saponins, Phenolic compounds, Flavonoids, Glycosides, Flavanol glycosides, Cardiac glycosides, Phytosterols, Fixed oils and fats and Gums and Mucilages according to standard methods [15].

Quantification of secondary metabolites

Quantification of total phenolics

The total phenolic content was calculated using technique [16]. Test tubes were filled with 50 microlitre triplicates of leaf extracts (20 mg/20 mL) and distilled water to 1 mL. Each tube received 0.5 mL Folin-ciocalteu phenol reagent (1:1 with water) and 2.5 mL sodium carbonate solution (20%) successively. After vortexing the reaction mixture, the test tubes were darkened for 40 min and the absorbance measured at 725 nm against the reagent blank. Results were reported as gallic acid equivalents after triple analysis.

Quantification of total tannins

After PVPP treatment, tannins were calculated [17]. After weighing 100 mg of PVPP in a 100 × 12 mm eppendrof tube, add 1 mL of distilled water and 1 mL of sample extracts. The contents were vortexed and frozen at 4°C for 15 min. Next, the sample was centrifuged at 4000 rpm for 10 min at room temperature to obtain the supernatant. The tannins would have precipitated with the PVPP, leaving this supernatant with only simple phenolics. Following the foregoing technique, the supernatant's non-tannin phenolic content was measured.

Tannins = Total phenolics - Non tannin phenolics

Quantification of total flavonoids

Leaf extract flavonoids were measured using technique [18]. Add 100 µL of plant extracts to separate test tubes and add 2 mL of distilled H_2O. A 2.5-mL distilled H_2O test vial was blank. Next, add 150 µL of 5% NaNO2 to each test tube and incubate at room temperature for 6 minutes. After incubation, 150 µL of 10% $AlCl_3$ was added to all test tubes, including the blank. All test tubes were incubated at room temperature for 6 min. After adding 2 mL of 4% NaOH to all test tubes, distilled water was added to 5 mL. After vortexing, all test tubes were left at ambient temperature for 15 minutes. Flavonoids caused a pink color that was measured at 510 nm. From the calibration curve: y = 0.0255x, R2 = 0.9812, where x was absorbance and y were quercetin equivalent (mg/g), total flavonoid content was estimated.

In vitro antioxidant activity

DPPH radical scavenging activity

The extracts' hydrogen donating or radical scavenging activity was measured using the stable radical DPPH [19]. The sample extracts (20-100 µL) were adjusted to 100 µL using methanol. The samples and standards (BHT and Rutin) were shaken briskly with 3 mL of a 0.004% methanolic DPPH solution. To generate the negative control, 100 µL of methanol was added to 3 mL of methanolic DPPH solution. Stand the tubes at 27°C for 30 min. The samples and control were measured at 517 nm against the methanol blank. Sample IC_{50} values indicate the concentration needed to block 50% of $DPPH^{\cdot}$ concentration, indicating radical scavenging activity.

ABTS radical cation scavenging activity

The total antioxidant activity was determined by ABTS radical cation scavenging assay by the method [20]. ABTS radical cation was produced by ABTS (stable radical) aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h. Prior to assay, ABTS solution was diluted in ethanol (1:89 v/v) to give an absorbance of 0.700 ± 0.02 at 734 nm. About 1 mL of diluted ABTS solution was added to about 30 µL sample solution and 10 µL of Trolox (final concentration 0–15 µM) in ethanol. A test tube containing 1 mL of diluted ABTS solution and 30 µL of ethanol served as the negative control. All the test tubes were vortexed well and incubated exactly for 30 min. at room temperature. After incubation the absorbance of samples and standards (BHT and Rutin) were measured at 734 nm against the ethanol blank. The results were expressed as the concentration of Trolox having equivalent antioxidant activity expressed as µM/ g sample extracts.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capabilities of sample extracts were evaluated using method [21]. Mix 900 µL of newly made, 37°C-incubated FRAP reagent with 90 µL of distilled water, 30 µL of test sample, and methanol (blank). BHT and rutin served as benchmarks. In a water bath, all test tubes incubated at 37°C for 30 min. Final reaction mixture dilution of test sample was 1/34. Mix 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$. $6H_2O$, and 25 mL of 0.3 M acetate buffer (pH-

3.6) to make the FRAP After incubation, the blue color's absorbance was measured at 593 nm against the reagent blank. Methanolic solutions with $FeSO_4.7H2O$ concentrations from 500 to 4000 µM were utilized to create the calibration curve. Equivalent Concentration was the antioxidant concentration that reduced ferric-TPTZ like 1 mM $FeSO_4.7H_2O$.

Superoxide radical scavenging activity

The experiment used extracts to scavenge superoxide radicals from riboflavin–light–NBT system to prevent formazan production [22]. A reaction combination of 50 mM sodium phosphate buffer (pH-7.6), 20 µg riboflavin, 12 mM EDTA, and 0.1 mg NBT was added to 100 µL sample solution, BHT, and rutin. Highlighting the reaction mixture with samples for 90 seconds initiated the reaction. The negative control was lit reaction mixture without sample. Absorbance was measured at 590 nm against the blank (unilluminated reaction mixture without sample) soon after illumination. Superoxide anion scavenging activity was estimated as:

Scavenging activity (%) = [(Control OD – Sample OD)/Control OD] × 100

Phosphomolybdenum assay

The green phosphomolybdenum complex production technique [23] measured sample antioxidant activity. In a test tube, 300 µL of methanol was used as the blank. Using 100 µL samples (1 mg/mL organic solvents) and standards (BHT and rutin), methanol was added to test tubes up to 300 µL. After adding 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), all test tubes were vortexed. Foil-covered test tubes were incubated at 95°C for 90 min. At 695 nm, the mixture's absorbance was measured against the reagent blank after cooling to room temperature. Laboratory findings were represented in milligrams of ascorbic acid equivalents (AAE)/g extract.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

The qualitative phytochemical screening was carried out in various extracts of leaves of *P. peepuloides* and *P. hymenophyllum* to identify the presence of major primary and secondary phytochemicals (Table 1). The results revealed that the primary metabolites such as carbohydrates, proteins and amino acids are present in all the extracts of both the leaf of the present study. The secondary metabolites such as alkaloids, saponins, phenol, flavonoid, glycoside, flavonos glycosides, cardiac- glycosides, phytosterols, fixed oils and fats, gums and Mucilages were found to be variously distributed in the selected leaf extracts. However, saponins were found to be absent in petroleum ether and chloroform extract of both the leaf samples. Flavonoid-glycosides and Gums and Mucilages were found to be absent in all extract of both the leaf samples.

Phytochemical properties of *Piper longum* and *Piper nigrum* leaves were evaluated [24] and they reported both plant species are rich in phytochemical constituents such as alkaloids, steroids, flavonoids etc. The sample of betel leaves collected from Tamil Nadu, India is known to contain steroids, tannins, proteins, amino acids, flavonoids, terpenoids, mucilage, volatile oil, saponin, carbohydrates, and fixed oil, but an absence of alkaloids [25]. It has been reported that high molecular weight compounds such as phenolics, tannins possess high ability in scavenging free radicals. They constituent to several drugs because of their astringent property. They are used in the treatment of several diseases such as haemorrhoids, diarrhoea, dysentery, leucorrhoea etc. [26].

Table 4 Phytochemical screening of *Piper peepuloides* and *Piper hymenophyllum* leaves

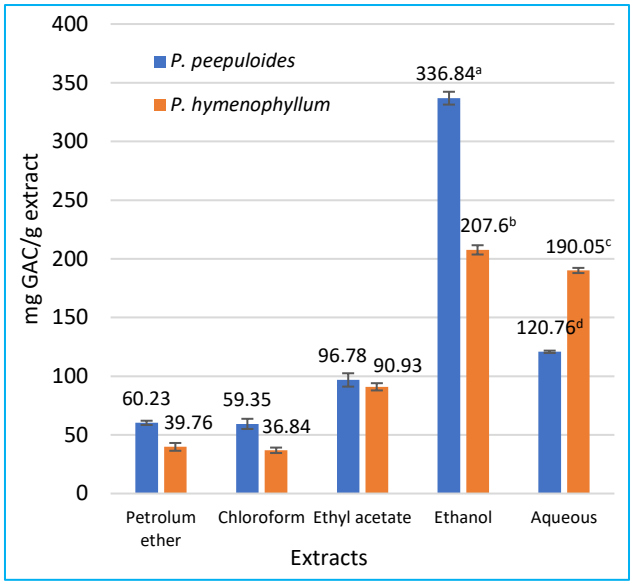
Sample	*Piper peepuloides*					*Piper hymenophyllum*				
Phytochemical	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Carbohydrates	+	+	++	++	+	+	+	++	++	+
Proteins	+	+	+	++	+	+	+	+	+	+
Amino acids	+	+	+	++	+	+	+	+	+	+
Alkaloids	+	+	++	+	+	+	+	++	+	+
Saponins	-	-	+	+	++	-	-	+	+	++
Phenol	++	+	++	+	+	+	+	++	+	+
Flavonoid	+	+	++	++	+	+	+	+	++	+
Glycosides	+	+	+	+	+	+	+	+	+	+
Flavonos glycosides	-	-	-	-	-	-	-	-	-	-
Cardiac glycoside	++	+	+	+	+	++	+	+	+	+
Phytosterols	+	++	++	++	+	+	++	++	+	+
Fixed oils and fats	++	+	+	+	+	++	+	++	+	+
Gums and mucilages	-	-	-	-	-	-	-	-	-	-

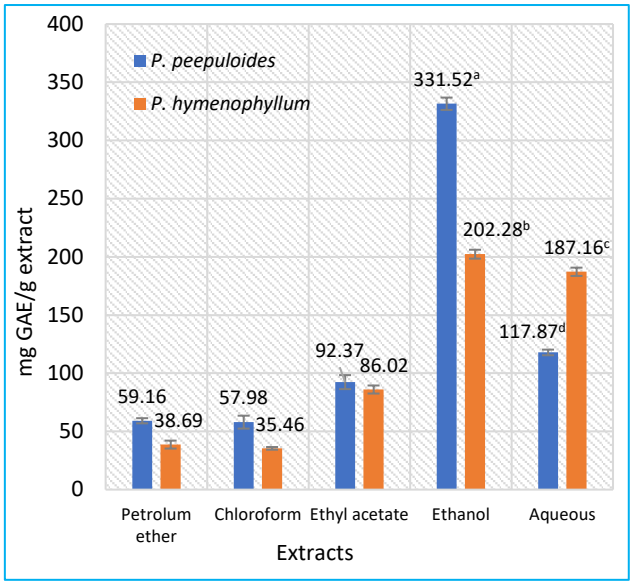
(+): Presence of chemical compound, (-): Absence of chemical compound
(+) < (++) < (+++): Based on the intensity of characteristic colour

Quantitative analysis of secondary metabolites

Determination of total phenolics and tannin contents

In this study, the total phenolics content is all the leaf extracts were estimated by Folin–Ciocalteu reagent method and the results are shown in (Fig 1-2). The total phenol content was expressed in Gallic Acid Equivalents (GAE). The highest amount of total phenolic was obtained from ethanol extract of *P. peepuloides* (336.84 mg GAE/g extract) and *P. hymenophyllum* (207.6 mg GAE/g extract) followed by *P. hymenophyllum* aqueous extract (190.05 mg GAE/g extract). The results clearly indicated that the *P. peepuloides* ethanol extract contains the highest total phenolic content when compared to *P. hymenophyllum***.** The tannins were found to be higher in ethanol extracts of both the leaf samples of (*P. peepuloides* 331.52 mg GAE/g extract and *P. hymenophyllum* 202.28 mg GAE/g extract) followed by aqueous, ethyl acetate, chloroform and petroleum ether extracts.

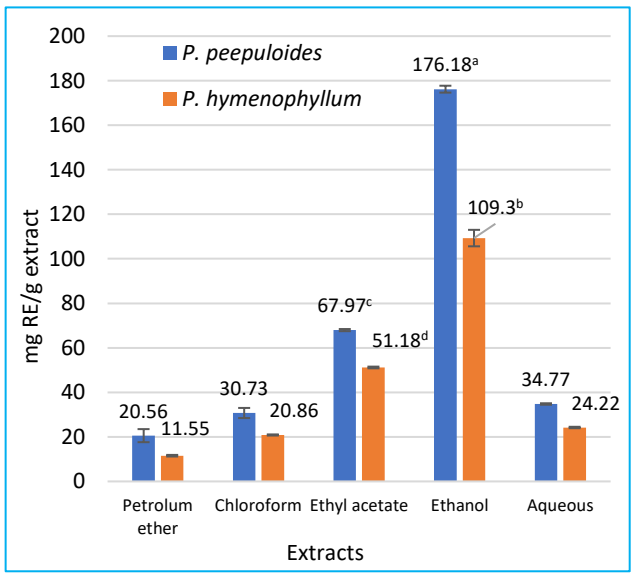




Values are mean of triplicate determination (n=3) ± standard deviation, GAE - Gallic acid equivalent
Statistically significant at $p < 0.05$ where $^{a} > ^{b} > ^{c} > ^{d}$

Fig 1 Total phenolics content of *P. peepuloides* and *P. hymenophyllum* leaf extracts

Fig 2 Total Tannin content of *P. peepuloides* and *P. hymenophyllum* leaf extracts



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

Fig 3 Total flavonoids content of *Piper peepuloides* and *Piper hymenophyllum* leaf extracts

Determination of flavonoid contents

The flavonoid content in different extract of *P. peepuloides* and *P. hymenophyllum* leaves were analyzed (Fig 3). The total flavonoid content was expressed in Rutin equivalents (RE). Among all the extracts, the ethanol extracts of *P. peepuloides* (176.18 mg RE/g) and *P. hymenophyllum* (109.3 mg RE/g) were found to have appreciable amount of flavonoid contents. The results revealed that the *P. peepuloides* ethanol extract contains the highest total flavonoid content.

Flavonoids such as catechin, quercetin and myricetin, and carotenoids, namely lutein and β-carotene was detected in significant concentration in black pepper. [27-28] reported 37.48 mg GAE/g and 3.01 mg/g quercetin equivalents/g in the methanol extract of *P. nigrum*. The total flavonoid and total phenolic contents of green peppers was found to be 1083.43 ± 8.24 mg CE/100 g and 1414.63 ± 10.56 mg CE/100 g, respectively [29].

In vitro antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activity of various extracts of *P. peepuloides* and *P. hymenophyllum* leaf are shown in (Fig 4). The IC_{50} value of all the extracts was determined, which depicted the concentration of the extracts required to scavenge 50% of DPPH free radical. Low IC_{50} value represents high antioxidant potential. IC_{50} value of ethanol extract of *P. peepuloides* was found to be 24.33 µg/mL. The result indicated that *P. peepuloides* leaf extract contains highest antioxidant activity than *P. hymenophyllum* (26.34 µg/mL) leaf. Among the extracts studied, ethanol extract showed potential antioxidant activity in both the leaf compared with other extract i.e aqueous, ethyl acetate, chloroform and petroleum ether. When compared with the natural antioxidant rutin (7.93 µg/mL) and synthetic antioxidant BHT (6.35 µg/mL) the studied leaf samples also possesses appreciable free radical scavenging activity.

Several authors have evaluated the radical scavenging activity of Piper species in different extract [30-32]. The reported that *P. officinarum* has high DPPH scavenging capacity and [33] have evaluated the radical scavenging activity in the ethanolic extract of *P. longum* and the IC_{50} value was 10µg/mL. [34-36] has worked on *P. guineense* reported that the IC_{50} value to range between 69.05 to 74.0 µg/mL. [37] have reported that *P. nigrum* and *P. refractum* have high DPPH radical scavenging activity. The IC_{50} values of DPPH antioxidant activity of the essential oils was found to be 44.16 and 22.88 mg/mL in Indigenous and Kerala cultivars, respectively. The antimicrobial activity of the essential oils showed good activity in both cultivars [37]. On comparing the previous reports of DPPH radical scavenging activities of various Piperaceae members, the selected plants *P. peepuloides* and *P. hymenophyllum* leaf extracts have promising radical scavenging activities, which might be due to the higher phenolic contents.

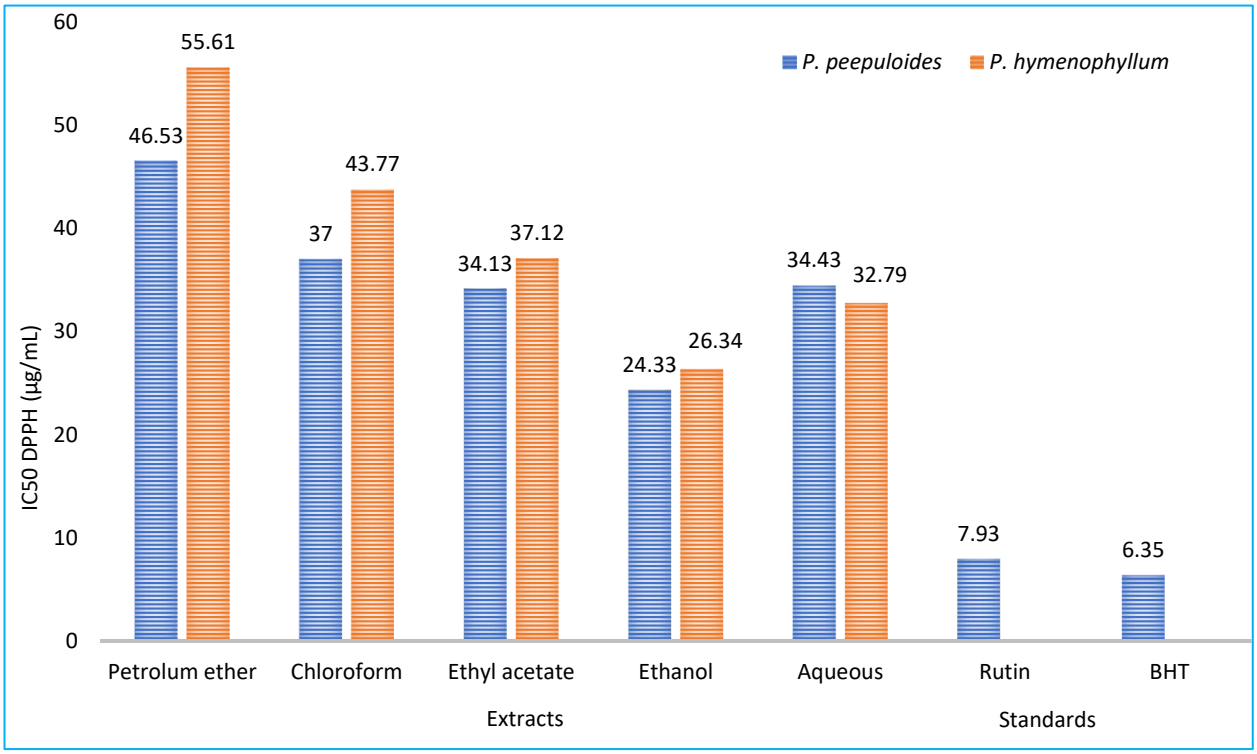


Fig 4 DPPH radical scavenging activity of *Piper peepuloides* and *Piper hymenophyllum* leaf extracts

ABTS cation radical scavenging activity

The TEAC (Trolox Equivalents Antioxidant Capacity) was measured using the improved $ABTS^{+}$ radical decolourisation assay. The results were expressed as µM Trolox Equivalents/g of extract. The results of ABTS cation radical scavenging activities of *Piper peepuloides* and *Piper hymenophyllum* leaf extracts are shown in (Table 2). The aqueous extract of the leaf showed higher radical scavenging activity *P. hymenophyllum* 68645.83 µM TE/g, followed by *P. peepuloides* ethanol extract (63611.11 µM TE/g). The standard natural antioxidant rutin (138125 µM TE/g sample) and synthetic antioxidant BHT was found to be 139583.3 µM TE/g extract.

Extraction procedures greatly affect the yield and biological activities of hydroxychavicol from *Piper betle* L. Four antioxidant assays were performed to evaluate the antioxidant activity of the extracts and pure hydroxychavicol. All the crude extracts and pure hydroxychavicol except for M3M showed remarkable ABTS scavenging activity. Meanwhile, the highest percentage of BCB inhibition was exhibited by M1 with 79.93% inhibition. However, it was not as effective as gallic acid (88.58% inhibition). The highest FRAP value was also demonstrated by M1 with the value of 11.45 µg gallic acid/ mg extract while the highest TAOC was exhibited by M3M with a value of 24.10 µg ascorbic acid/ mg extract [38].

Table 2 $ABTS^{\bullet+}$ cation redical scavenging activity of *P. peepuloides* and *P. hymenophyllum* leaf Extracts (µM TE/g extract)

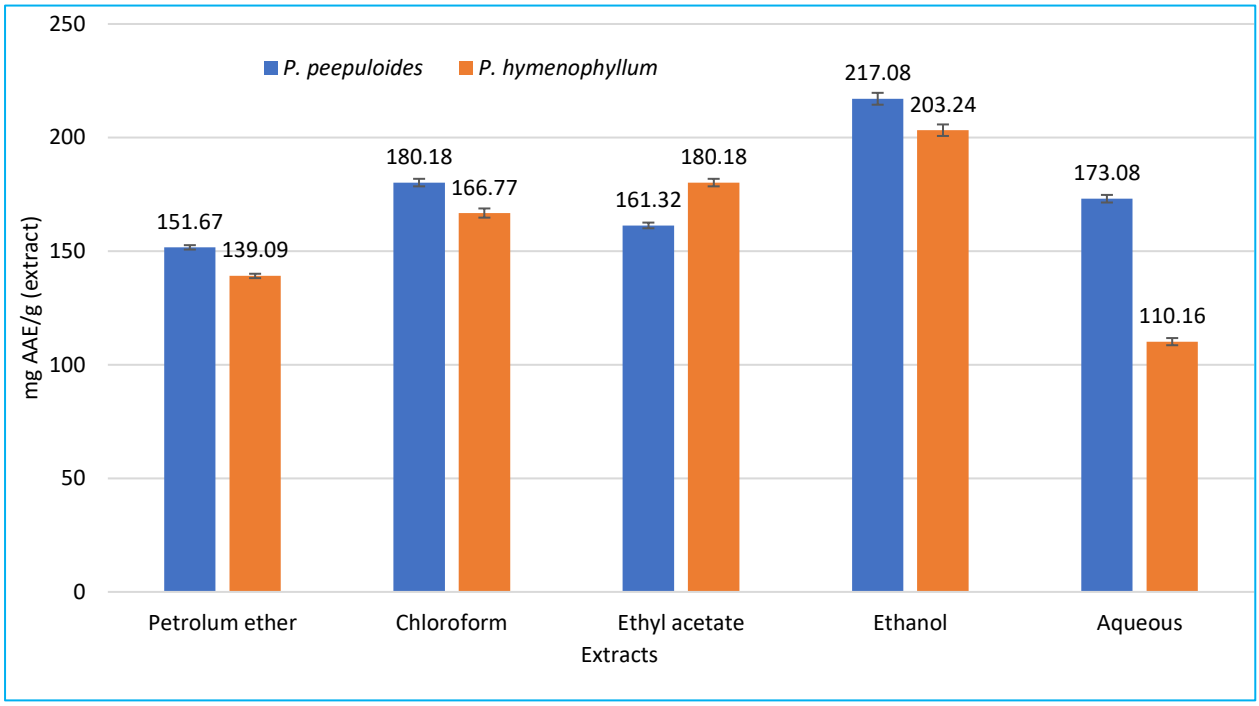
Sample	Extracts	ABTS scavenging Activity (µM TE/g extract)	
		Piper peepuloides	*Piper hymenophyllum*
Leaf	Petroleum ether	35312.5 ± 813	30625 ± 275
	Chloroform	43020.83 ± 1900	40729.17 ± 375
	Ethyl acetate	58055.56 ± 216.84	47847.22 ± 60.14
	Ethanol	63611.11 ± 394^{d}	62291.67 ± 104.16
	Aqueous	52777.78 ± 910	68645.83 ± 275.59^{c}
Standards	Rutin	138125 ± 416^{b}	
	BHT	139583.3 ± 416^{a}	

Values are mean of triplicate determination (n=3) ± standard deviation, TE - Trolox Equivalents
Statistically significant at $p < 0.05$ where a > b > c > d

Phosphomolybdenum reduction assay

The phosphomolybdneum assay is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate/Mo (V) complex and was measured at absorption at 695 nm. The total antioxidant capacity of different extracts of *P. peepuloides* and *P. hymenophyllum* leaf were analyzed and the results were shown in Fig. 5. The better antioxidant capacity was observed in ethanol extract of *P. peepuloides* (217.08 mg AAE/g extract) and *P. hymenophyllum* (203.24 mg AAE/g extract), followed ethyl aceatate and chloroform extract of *P. hymenophyllum* (180.18 and 180.18 mg AAE/g extract). The other extracts also showed good antioxidant activity.

The ethanolic fractions of *Piper cubeba* and has reported to have high antioxidant activity. ABTS radical scavenging assay in *Piper nigrum* seed which ranged from 0.76-79.12 µg/ml [39-41]. When compared to other Piper the results of other Piperaceae members have been identified to have low scavenging property than the selected plant sample *Piper mullesua* and *Piper wightii.*



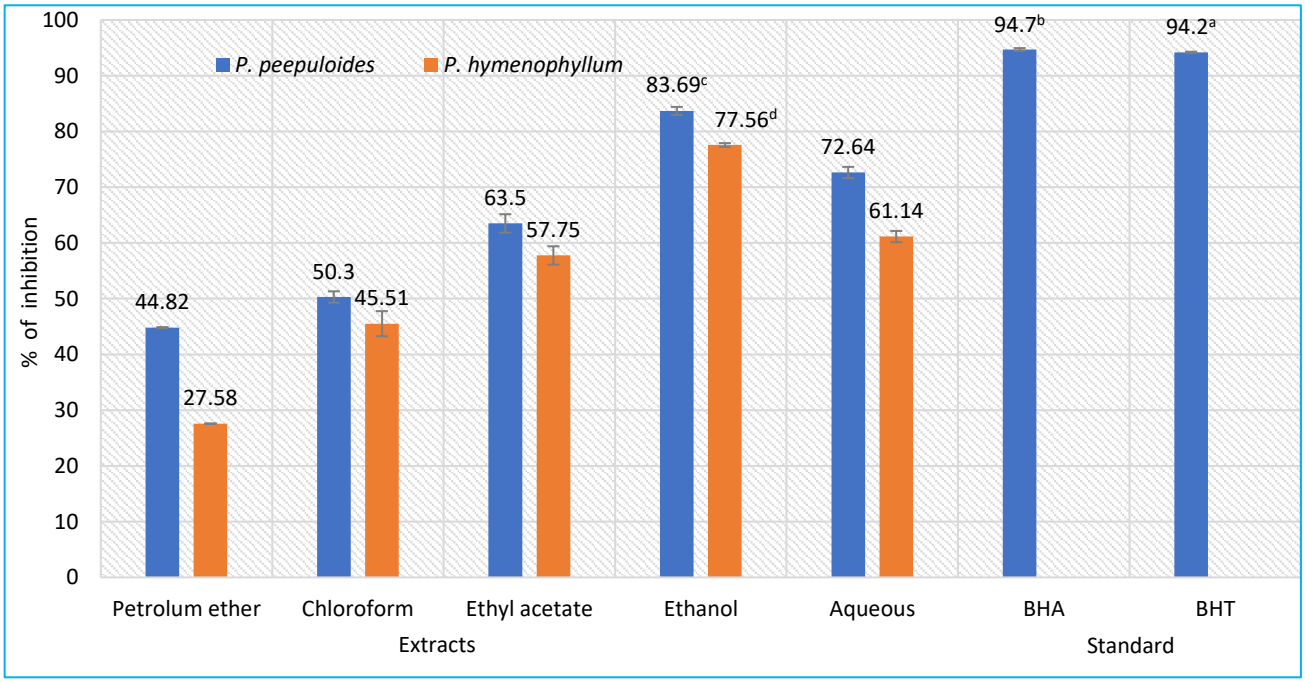
Values are mean of triplicate determination (n=3) ± standard deviation; Statistically significant at $p < 0.05$ where $^{a} > ^{b} > ^{c} > ^{d}$

Fig 5 Phosphomolybdenum assay of *Piper peepuloides* and *Piper hymenophyllum* leaf extracts

Superoxide radical scavenging activity

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be produced *in vivo* result in the formation of Hydrogen peroxide (H_2O_2) via dismutation reaction. The results of superoxide anion scavenging activities of leaf extracts of *Piper peepuloides* and *Piper hymenophyllum* are shown in (Fig 6). The extracts were found to be an efficient scavenger of superoxide radical generated in riboflavin- NBT- light system *in vitro*. The scavenging activities of ethanol extract of *Piper peepuloides* were found to be 83.69% followed by ethanolic extract of *Piper hymenophyllum* 77.56%. The ethanol extracts of both the samples showed good free radical scavenging activity to that of BHT (94.2%) and BHA (94.7%).

There are very less reports available on SO scavenging properties of the related piper species. [42] have reported P. nigrum and Myristica fragrans seed ethanolic extract to have similar radical scavenging activity as of the selected plants. [43] has worked on *Piper longum* which have high radical scavenging activity and the plant contributes several amino acids such as valine, proline, isoleucine and leucine which highly contribute to the scavenging of free radicals against superoxide ions, which have been identified to have the high antioxidant capacity as that of the selected plant sample *Piper peepuloides* and *Piper hymenophyllum*.



Values are mean of triplicate determination (n=3) ± standard deviation; Statistically significant at $p < 0.05$ where $^{a} > ^{b} > ^{c} > ^{d}$

Fig 6 Superoxide radical scavenging activity of *Piper peepuloides* and *Piper hymenophyllum* leaf extracts

CONCLUSION

The present study highlights remarkable Qualitative Phytochemical Screening, secondary metabolites and antioxidant properties of selected *Piper* species, among which the versatile plant, *Piper peepuloides* and *Piper hymenophyllum* stood as the best source of various types of compounds with diverse chemical structure. In future, researchers have a promising scope in isolation, identification and characterization of active chemical components, which yields a better understanding of the bioactive products, subsequently helping in the management of disease.

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