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Research Article

EVALUATION OF PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF ETHNOMEDICINAL PLANT PSYCHOTRIA NILGIRIENSIS DEB. & GANG.

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ABSTRACT

Objective: To investigate phytochemical properties, free radical scavenging and antimicrobial activity of medicinal plant *Psychotria nilgiriensis* fruit and root extracts.

Methods: Dried, powdered fruit and root were extracted by Soxhlet apparatus with successive solvent extraction method. The filtrates were used for preliminary phytochemical analysis, radical scavenging assays (DPPH, ABTS and FRAP) and antimicrobial studies (Agar well diffusion method).

Results: Preliminary phytochemical analysis clearly showed the *P. nilgiriensis* fruit contains notable amount of phenolics, tannin and flavonoids. Acetone extract of fruit showed significant antioxidant scavenging activity in DPPH (20.0 µg/ml), ABTS (41343.51 µM TE/g extract) and FRAP (4713.33 mM Fe (II)/mg extract) assays. Acetone extract of fruit showed highest inhibitory activity (IZ 22.6 mm) against *K. pneumonia* at 200 mg/mL. The lowest inhibitory activity (IZ 2.0 mm / 100mg/mL) against bacterial species were showed by hot water extract of root against *S. pneumoniae*. Except the hot water extract all other extracts were resist the *Aspergillus* sp at different inhibitory zone levels from 0.5 to 14.0 mm depending upon their concentration.

Conclusion: The acetone extract of *P. nilgiriensis* fruit and root contains notable chemical compounds that are responsible for its antioxidant and antimicrobial activity.

Keywords: *Psychotria nilgiriensis*, Phenolics, Antioxidant, Antimicrobial

INTRODUCTION

Medicinal plants are known to contain innumerable biologically active compounds. They have continued to play a dominant role in the maintenance of human health since ancient times. Several active compounds have been revealed from plants on the basis of Ethnobotanical information and used directly or patented drugs. Increasing resistance pattern of microorganisms to most currently used antimicrobial drugs has increased the scientific interest to explore the plant source secondary metabolites to formulate new therapeutic drugs. According to World Health Report of infectious diseases (2000), overcoming antibiotic resistance is one of the major issues of the WHO for the present millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management [1].

The imbalance between antioxidants and free radicals in our body is responsible for most of our maladies. Reactive oxygen species (ROS) are continuously produced during cell metabolism and under normal condition they are scavenged and converted to non-reactive species by different intracellular enzymatic and non-enzymatic antioxidant system [2]. Thus, a practical way to control the diseases to enhance the dietary intake of fresh vegetables, fruit and greens, which are affluent sources of antioxidants.The synthetic antioxidants such as BHT, BHA, TBHQ and natural antioxidants like Tocopherol and phenolic acids are used to protect against free radical related oxidative damage. Although synthetic antioxidants have shown, good efficiency their use has been limited because of their detrimental effect on human health [3].

Throughout the history of mankind, many infectious diseases have been known to be treated with herbal remedies. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs for the treatment of infectious diseases. Hence much attention has been paid recently to the biologically active compounds derived from plants used in herbal medicine.

The genus *Psychotria* of Rubiaceae contains medicinally valuable indole alkaloids namely psychotridine and brachycerine. Ethnobotanical studies on species of *Psychotria* resulted in the discovery of a set of novel bioactive mono indole alkaloids (MIAs), some of them with clear pharmaceutical potential. *Psychotria nilgiriensis* is a large shrub or small tree 4m in height and grows in high elevation evergreen forests between 2000 and 2300 m. *P. nilgiriensis* tender fruit is consumed along with honey for its action against rheumatism [4]. It is being used by Kanikkar tribes of Kalakad, Mundanthurai at Tirunelveli district and Irula tribes of Thottabetta at Nilgiris district (Western Ghats), Tamil Nadu. Even though the plant has been reported for its Ethnobotanical uses, there are no scientific reports were available regarding on antimicrobial aspect. Therefore, this study was aimed to investigate the comparative analyses of phytochemical, radical scavenging and antimicrobial activities of different solvent extraction from the *P. nilgiriens* fruit and root.

Collection and identification of plant materials

The fresh plant parts of *P. nilgiriensis* were collected from Thottabetta, Nilgiris, Tamil Nadu during the month of February to April, 2011.The collected plant material was identified and their authenticated by comparing the voucher specimen at the herbarium of Botanical survey of India, Southern Circle - Coimbatore, Tamil Nadu. Freshly collected plant materials was cleaned to remove adhering dust and then dried under shade. The dried sample were powdered and stored in air tight containers separately for further studies.

Chemicals

Solvents, Antioxidant and Antimicrobial Chemicals are purchased from Sigma, Merck and Hi-media. All other reagents used were of analytical grade. All the microorganisms used for the studies were procured from P.G.P. college of Arts and Science, Namakkal.

Successive solvent extraction

The air dried, powdered plant material was extracted in Soxhlet extractor successively with petroleum ether, chloroform, acetone and methanol. Each time before extracting with the next solvent, the material was dried in hot air oven below 40˚C. Finally, the material was macerated using hot water with occasional stirring for 48 h and the water extract was filtered. The different solvent extracts were concentrated by rotary vacuum evaporator and then air dried. The dried extract obtained with each solvent was weighed. The

percentage of yield was expressed in terms of air dried weight of plant material and the extracts were freeze dried and stored in desiccators. The extracts thus obtained were used directly for the estimation of total phenolics and also for the assessment of antioxidant potential through various biochemical assays.

Extract recovery percentage

The extracts were dried after successive solvent extraction and the recovery percentage were calculated as follows:

Recovery % = Extract weight /Plant sample weight (g) × 100

Qualitative Phytochemical Screening

The fruit and root extracts of *P. nilgiriensis* 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 [5].

Determination of total phenolic and tannin contents

The total phenolics of the plant extracts were determined by Folin Ciocalteu method. Using the same extract the tannins were estimated after treatment with PVPP. The amount of total phenolics and tannins were calculated as the Gallic acid equivalents (GAE) as described by Siddhuraju and Becker [6].

Determination of total content of flavonoids

The total flavonoid content was determined by the aluminium chloride method [7]. Rutin was used as a reference for the calibration curve. The absorbance of the reaction mixture was measured at 415 nm in UV spectrophotometer (Shimadzu – UV 1800, Japan).

***In vitro* antioxidant activity**

Estimation of Free Radical Scavenging Activity by DPPH Method

The antioxidant activities of the crude extracts were evaluated by their ability in quenching the stable free radical DPPH, according to the method described by Blois [8]. The DPPH quenching ability was expressed as IC_{50} (the extract concentration (µg/mL) required to inhibit 50% of the DPPH in the assay medium).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of extracts were estimated according to the procedure described by Pulido *et al*. [9]. Freshly prepared FRAP reagent was incubated at 37°C. To the 90 µl of distilled water and 30 µL of test sample or methanol (for the reagent blank), 900 µL of FRAP reagent was added. The prepared test samples and reagent blank were again incubated in water bath at 37°C for 30 min. The final dilution of the test sample in the reaction mixture was 1/34. At the end of incubation, the absorbance readings were taken immediately at 593 nm. Results were expressed in ascorbic acid equivalents.

$ABTS^{\bullet+}$ scavenging activity

The total antioxidant activity of the samples was measured by ABTS cation radical decolourization assay [10]. 7 mM ABTS aqueous solution was added with 2.4 mM potassium persulfate and this mixture was incubate at dark for 12–16 hours to produce $ABTS^{\bullet+}$. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 25°C 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. 1 mL of diluted ABTS solution was mixed with 30 µL of ethanol served as the negative control. All the test tubes were vortexed well and incubated exactly for 30 minutes 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

Bacterial and fungal species

The following Gram-positive species: *Bacillus subtilis*, *Staphylococcus aureus, Streptococcus pneumoniae* and Gram-negative species: *Salmonella typhi*, *Klebsiella pnemoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and two fungal species: *Asperigillus niger*, *Aspergillus fumigates* were used for antimicrobial studies.

Anti-microbial studies

Antibacterial and antifungal activities of plant extracts were investigated by the agar well diffusion method [11]. Fresh bacterial cultures were prepared and used to inoculate 50 ml of Muller-Hinton broth that was incubated at 35°C for 18 h. Overnight broth cultures were prepared, adjusted in peptone-physiological salt solution (1 g peptone and 8.5 g/L NaCl) to yield approximately 106 bacteria/mL. All the fungal cultures were inoculated onto Sabouraud dextrose agar plates. The agar plates for bacteria and fungus were prepared in 90 mm Petri dishes with 22 mL of agar medium giving a final depth of 3 mm. The agar was allowed to set and harden and required numbers of holes were made using a sterile cork borer. Agar plugs were removed different cork borers were used for different test organisms. Different solvent extracts of fruit and root were loaded into well bored in inoculated agar surfaces at different concentration (100, 200 and 250 mg/mL). Respective solvents were used as negative control. Chloramphenicol (for bacteria) and Streptomycin (for fungi) 20 µL were used as positive control. The concentration of the standard drug used for the antimicrobial study was 10 mg/mL. The plates inoculated with bacteria were aseptically incubated at 37°C for 18-24 h, while those inoculated with fungus were incubated at 28°C for 48h. The antimicrobial activity was estimated by zone of inhibition (IZ) between the edge of the well and the edge of the inhibition area (mm). Each test was performed in triplicate and the results were shown as means.The whole experiment was performed under strict aseptic conditions.

RESULTS

Extract yield percentage

The yield percentage of *P. nilgiriensis* was shown in Table 1. The Acetone extract of fruit showed higher amount of yield 16.30% followed by the methanol extract of root showed 12.64% yield. On the other hand, chloroform extracts of the plant, showed lower recovery percentage compared to other solvent extracts.

Table 1: Extract Yield Percentage of *P. nilgiriensis*

Solvents	Recovery % (g/100 g of dried powder)	
	Fruit	Root
Petroleum Ether	3.89	0.68
Chloroform	2.8	0.54
Acetone	16.30	7.13
Methanol	8.05	12.64
Hot Water	6.79	5.42

Preliminary phytochemical screening

Preliminary phytochemical screening was done as an important initial step to determine the phytochemical constituents in *P. nilgiriensis* which helped to make a clear approach towards the objectives of study. Phytochemical analysis shows considerable amount of primary metabolites in *P. nilgiriensis* fruit and root. The secondary metabolites such as alkaloids, saponins, phenolic compounds, tannins, flavonoids, phytosterol and glycosides were found to be variously distributed in the plant extracts. The results are tabulated below in Table 2.

Quantification of total phenolics, tannins and flavonoids

Acetone extract of *P. nilgiriensis* fruit has highest phenolic content (505.74 mg GAE /g extract) when compared to other extracts. The least phenol percentage was obtained from petroleum ether extract (25.91 mg GAE/g extract). The acetone extract of fruit also showed maximum tannin content (460.78 mg GAE/g extract) followed by acetone extract of root (434.73 mg GAE /g extract). The least amount of tannin content was observed in petroleum ether extract

of fruit (1.54 mg GAE/g extract). The estimation of total flavonoid content in acetone extract of fruit revealed maximum amount (67.78 mg RE/g extract) and it followed by acetone extract of root (33.67 mg RE/g extract). While the least amount of flavonoid content was recorded in petroleum ether extract of fruit (1.52 mg RE /g). The results of total phenolics, tannin and flavonoids has been summarised in Table 3.

Table 2: Phytochemical screening of *P. nilgiriensis* Fruit and Root extracts

Plant Constituents	Fruit	Root
Carbohydrates	+++	+
Proteins	+	+
Amino acids	++	+
Alkaloids	+++	++
Saponins	++	++
Phenolic compounds	+++	+++
Tannins	++	++
Flavonoids	+++	+++
Glycosides	+	++
Flavonol Glycosides	++	+
Cardiac glycosides	+++	+++
Phytosterols	+++	++
Fixed oils and fats	+	+
Gums and mucilages	-	-

(+): Presence of chemical compound, (-): Absence of chemical compound

(+) < (++) < (+++): Based on the intensity of characteristic colour

Table 3: Total Phenolic, Tannin and Flavonoid contents of *P. nilgiriensis*

Extracts	Phenolic (mg GAE/ g extract)	Tannin (mg GAE/ g extract)	Flavonoids (mg RE/ g extract)
PNFP	25.91 ± 1.35	1.54 ± 0.64	1.52 ± 0.09
PNFC	77.31 ± 5.88	30.81 ± 4.25	2.22 ± 0.38
PNFA	505.74 ± 82.38[a]	460.78 ± 80.70[a]	67.78 ± 5.03[a]
PNFM	225.91 ± 12.34	182.63 ± 15.00	33.33 ± 0.12[b]
PNFHW	48.04 ± 6.12	5.18 ± 6.73	3.24 ± 0.03
PNRP	39.64 ± 4.23	3.92 ± 3.48	1.97 ± 0.23
PNRC	77.45 ± 4.31	20.31 ± 4.92	2.05 ±0.07
PNRA	490.62 ± 28.94[b]	434.73 ± 29.11[b]	33.67 ± 4.25[b]
PNRM	322.69 ± 13.36[c]	281.79 ± 14.93[c]	5.51 ± 0.23
PNRHW	162.46 ± 8.66	141.46 ± 155.27	2.04 ± 0.01

PNFP - Psychotria nilgiriensis fruit petroleum ether, PNFC- Psychotria nilgiriensis fruit chloroform, PNFA - Psychotria nilgiriensis fruit acetone, PNFM - Psychotria nilgiriensis fruit methanol, PNFHW - Psychotria nilgiriensis fruit hot water, PNRP - Psychotria nilgiriensis root petroleum ether, PNRC- Psychotria nilgiriensis root chloroform, PNRA - Psychotria nilgiriensis root acetone, PNRM - Psychotria nilgiriensis root methanol, PNRHW - Psychotria nilgiriensis root hot water

Values are mean of triplicate determination (n=3) ± standard deviation, GAE- Gallic Acid Equivalents, RE- Rutin Equivalents

a>b>c - Statistically significant at P<0.05

Antioxidant assays

DPPH radical scavenging activity

The free radical-scavenging activities of different parts of *P. nilgiriensis* samples along with standards such as α-Tocopherol, rutin, BHA and BHT were determined by the DPPH radical scavenging assay and the results were showed in Table 4. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. The colour change from purple to yellow is visually evident. A lower value of IC_{50} (Inhibitory concentration at 50%) indicates a higher antioxidant activity. The highest free radical scavenging activity was exerted by acetone extract of fruit (20.0µg/ml).

ABTS cation radical scavenging activity

The results of $ABTS^{\cdot+}$ scavenging activities of fruit and root extracts of *P. nilgiriensis* were shown in Table 4. The results were expressed as µmol Trolox equivalent /g dry weight of plant material. The acetone extract of fruit showed higher radical scavenging activity (41343.51 µM TE/g extract) when compared to that of other solvent extracts. However, the fruit showed to appreciable total antioxidant activity in most of its solvent extracts. The higher scavenging activity of root was observed in its acetone extract (35437.30 µM TE/g extract).

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing capacity of *P. nilgiriensis* extracts was showed Table 4. The acetone extract of fruit has higher (4713.33 mM Fe (II)/mg) and least in petroleum ether extract of fruit (85.56 mM Fe (II)/mg). Among the root extracts highest ferric reducing capability was showed by acetone extract (4696.67 mM Fe (II)/mg).

Table 4: DPPH, ABTS and FRAP Assay results of *P. nilgiriensis*

Extracts	DPPH IC_{50} (µg/ml)	ABTS (µM TE/ g extract)	FRAP (mM Fe (II)/ mg extract)
PNFP	83.7±18.55	173.47 ± 14.22	85.56 ± 1.92
PNFC	73.4±53.20	712.12 ± 26.04	129.21 ± 11.76
PNFA	20.0±22.63	41343.51±58.46[a]	4713.33 ± 2.89[a]
PNFM	51.6±13.5	4775.60 ±88.27	1411.48 ±1.28
PNFHW	91.3±34.56	1427.62 ± 74.92	516.35 ± 13.04
PNRP	78.0±22.85	425.92 ± 46.01	163.65 ± 3.61
PNRC	98.7±50.86	975.37 ± 39.28	316.03 ± 26.56
PNRA	36.3±48.89	35437.30 ± 101.25[b]	4696.67 ± 32.83[b]
PNRM	69.7±11.43	6797.21 ± 50.96[c]	2255.56 ±90.74[c]
PNRHW	108.6±72.54	1015.87 ± 67.22	294.29 ± 7.84

Values are mean of triplicate determination (n=3) ± standard deviation, IC_{50}– Inhibitory Concentration at 50%, TE – Trolox Equivalent

a>b>c - Statistically significant at P<0.05

Table 5: Antibacterial Screening of *P. nilgiriensis* Fruit and Root Extracts

Extracts (200µg/ml)	Zone of Inhibition (mm)						
	Gram Positive Bacteria			Gram Negative Bacteria			
	B. subtilis	*S. aureus*	*S. pneumoniae*	*S. typhi*	*K. pnemoniae*	*E. coli*	*P. aeruginosa*
PNFP	5.8	4.9	6.9	11.6	9.8	11.4	8.9
PNFC	7.9	11.6	9.5	12.9	12.2	15.4	6.4
PNFA	18.6	21.5	14.8	17.5	22.6	13.8	13.9
PNFM	12.8	11.6	8.6	15.8	19.0	9.6	17.2
PNFHW	2.2	2.9	4.5	3.5	2.8	5.0	3.8
PNRP	3.6	6.2	5.7	10.2	7.9	9.4	6.9
PNRC	8.6	10.8	7.9	10.8	9.2	13.4	9.4
PNRA	9.0	16.8	11.5	13.5	16.6	13.8	14.7
PNRM	8.6	17.4	14.6	9.5	18.6	14.3	13.1
PNRHW	4.1	3.8	2.0	2.6	2.4	4.3	4.0
Standard Chloramphenicol (10µg/ml)	18.6	11.5	16.8	22.5	13.9	19.4	17.3

Table 6: Antifungal activity of *P. nilgiriensis* Fruit and Root Extracts

Extracts (200µg/ml)	Zone of Inhibition (mm)	
	Aspergillus niger	*Aspergillus fumigates*
PNFP	3.4	5.5
PNFC	2.8	4.3
PNFA	6.4	9.7
PNFM	14.0	10.6
PNFHW	0.5	1.2
PNRP	2.5	4.8
PNRC	1.6	1.1
PNRA	5.4	6.1
PNRM	11.5	7.9
PNRHW	0.3	-
Streptomycin (10µg/ml)	22.9	19.3

Antimicrobial activity

Antimicrobial activity of the plant extracts showed diverge degree of antimicrobial activity against the bacterial and fungal species (Table 5 & 6). Antimicrobial activity with zone of inhibition between the edge of the well and the edge of the inhibition area (IZ) were differed according to the tested organism. The antibacterial activities of the acetone and methanol extracts were compared favourably with that of standard antibiotics (Chloramphenicol and Streptomycin) and have appeared to be broad spectrum as its activities were independent on gram reaction. The acetoneextract (IZ 7 – 22.6 mm) was found to be more effective than the methanol extract (IZ 5.0 – 19.0mm) against all the organisms. The minimal antimicrobial activity was showed by hot water extract with IZ ranging between 1.0 and 6.0 mm for different microbes tested.Most probably the extracts were showed significant inhibitory zone against gram negative bacteria when compared to gram positive. Acetone extract of fruit showed highest inhibitory activity (IZ 22.6 mm) against *K. pneumonia* at 200mg/mL. The lowest inhibitory activity (IZ 2.0 mm / 100mg/mL) against bacterial species were showed by hot water extract of root against *S. pneumoniae*. Except the hot water extract all other extracts were resist the *Aspergillus* sp at different inhibitory zone levels from 2.0 to 14.0 mm depending upon their concentration.

DISCUSSION

Different solvents have been reported to have the capacity to extract different phytoconstitution depending on their solubility or polarity in the solvents [12]. According to the earlier reports, *P. nilgiriensis* extracts yield percentage was higher in acetone and methanol.

The preliminary phytochemical analysis of *P. nilgiriensis* showed significant level of secondary metabolites. Alkaloids, saponin, tannin, flavonoids glycosides were found in higher level in fruit. Primary assay confirm the high value of phenolic content in fruit and root. Several comprehensive works have been done on the effects of phenolic compounds on total antioxidants [13]. Fruit and root extracts were showed considerable amount of flavonoids. Medicinal plant origin flavonoids were found to possess analgesic and/or anti-inflammatory effects. Fruit extract exhibited high amount of colour reaction which confirmed the presence of alkaloids in higher level. Acetone and methanol extract of root showed moderate amount of alkaloid. Several *Psychotria* species have shown the presence of pyrrolidino indoline alkaloids. Alkaloids are very good analgesic agent and it have persuasive effect on inflammation.

Saponins are glycosides of triterpenes and steroids, are commonly occur in higher plants. Saponins exhibit a variety of biological activities and are widely used in foods and medicines. From the results of *P. nilgirensis* plant extracts ensure the presence of secondary metabolites such as phenolics, sterols, triterpenes and alkaloids which may possibly act as a good natural antioxidant.

Quantification of total phenolics

Phenolics substances have been shown to be responsible for the antioxidant activity of plant materials. Phenols are secondary metabolites in plants and are known to possess a wide range of therapeutic uses. The enrichment of phenolics compounds within plant extracts are correlated with their enhanced antioxidant activity [14]. The scavenging ability of the phenols is mainly due to the presence of hydroxyl groups. Total phenolics in acetone extract of fruit (505.74 mg GAE/g extract) were found to be higher than acetone extract of root (216 mg GAE/g extract). Dorman *et al*., [15] has been reported the strong relationship between total phenolic content and antioxidant activity in fruits, vegetables and grain products. In general, phenolic compounds are potentially effective against several microorganisims including many fungal pathogens [16]. Therefore, the higher phenolic content in acetone extract of fruit might be responsible for the enhanced antioxidative and antimicrobial activities.

Quantification of tannins

The total tannins were found to be higher in acetone extract of *P. nilgiriensis* fruit (460.78 ± 80.70 mg GAE/ g extract) and root (434.73 ± 29.11 mg GAE/ g extract). Recently, it has been reported that the high molecular weight phenolics such as tannins have more ability to reduce or scavenge free radicals. Lopez *et al*. [17] have also reported a correlation between antioxidant properties and total tannin content in *Canavailia cathartica* extracts. Tannins inhibit the absorption of minerals such as iron which may, if prolonged, lead to anaemia. This is because tannins are metal ion chelators [18] and tannin-chelated metal ions are not bioavailable. Tannins only reduce the bioavailability of plant sources of iron, also known as non-heme. Tannins interfere with iron absorption through a complex formation with iron when it is in the gastrointestinal lumen which decreases the bioavailability of iron. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders suchas diarrhea and dysentery [19]. Hence, the tannin content in *P. nilgiriensis* could show good chelation of Fe ion in antioxidant assays.

Quantification of flavonoids

Flavonoids are large compounds occurring ubiquitously in food plants. Flavonoids are a class of secondary plant phenolics with significant antioxidant properties [20]. Among bioactive compounds naturally occurring phenolic flavonoids have gained a particular interest because of their broad pharmacological activity. Putative therapeutic effects of much traditional medicine may be ascribed to the presence of flavonoid [21]. In our study, the flavonoid showed a significant positive correlation with the antioxidant activity of the plant extracts. The highest levels of total phenolic and flavonoid contents were found in acetone extract of *P. nilgiriensis* fruit (67.78 ± 5.03 mg RE/g),while flavonoid contents of root and fruit petroleum ether extracts were low (1.97 ± 0.23 and 1.52 ± 0.09 mg RE/g respectively). Flavonoids occur as glycosides and contain several phenolic hydroxyl groups on their ring structure. Many flavonoid are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups. The results revealed that the acetone extract of fruit contain significant level of flavonoid content than other parts of the same plant. Hence, presence of flavonoids supports that *P. nilgiriensis* can have antioxidant and other medicinal property.

DPPH radical scavenging assay

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. This method was introduced as an easy and accurate method for use in fruit and vegetable juice extracts. Therefore, numerous studies on antioxidants present in plants have been conducted using the DPPH assay, including fruits, vegetables, medicinal plants, cereals, spices and leaves [22].The antioxidants donate hydrogen to free radicals, leading to non-toxic species and therefore to inhibition of the propagation phase of lipid oxidation. Compare to other solvent extracts of the *P. nilgiriensis* acetone extract of fruit (20.0µg/ml), showed spectacular value in DPPH assay and IC_{50} value of this acetone extract was unconditionally significant than the standards α- tocopherol, rutin and BHT. Hot water extract of root enter the least value 108.6 µg/ml in DPPH radical scavenging activity. The acetone extract of *P. nilgriensis* fruit and root were possibly contained some substrates, which were electron donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction. The presence of flavonoids in the plants is likely to be responsible for the free radical scavenging effects is well documented [23]. Flavonoid content profile of this plant may be endorsed to scavenge the free radicals. The antioxidant potential of the plant to scavenge free radicals was ensured in the DPPH assay.

$ABTS^{\cdot+}$ assay

The TEAC (Trolox Equivalents Antioxidant Capacity) was measured using the improved $ABTS^{\cdot+}$ decolourisation assay, one of the most frequently employed methods for antioxidant capacity, which measures the ability of a compound to scavenge $ABTS^{\cdot+}$. Acetone extract of *P. nilgiriensis* fruit showed significant result (41343.51 µM TE/g extract) against ABTS free radical. Acetone extract of root (35437.30 µM TE/g extract) also confirm moderate result in radical scavenging and ensure the free radical scavenging activity. Petroleum ether extract of fruit (173.47 µM TE/g extract) failed to produce considerable result against ABTS cation radical. Low polarity of the solvent may be reason for the poor result in petroleum either extracts. Earlier reports of *Psychotria* sp. showed tremendous result in ABTS assay. The extensive investigations on antiradical and antioxidant activities of small phenolics including flavonoids and phenolic acids have been reported [24].

FRAP assay

The ferric reducing ability of the acetone extracts of *P. nilgiriensis* revealed excellent FRAP activity. Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom. The acetone extract of fruit revealed significant value (4713.33 mM Fe (II)/ mg extract) of ferric reducing power and the petroleum ether extract of fruit resulted least value (85.56 mM Fe (II)/ mg extract). Ferric reducing antioxidant power of the plant showed greater variability according to the part used and solvent. Halvorsen *et al*. [25] suggested most of the secondary metabolites are redox-active compounds that will be picked up by the FRAP assay. Hajimahmoodi *et al*., [26] has been reported different tea sample shad showed notable ferric reducing ability and antioxidant power which was highly correlated to phenolic, flavonoid and anthocyanidin contents of the tea samples.

Antimicrobial activity

Drugs derived from natural sources are efficient in management of infectious diseases at same time they mitigating many of the side effects that are often tied with synthetic antimicrobial drugs. Antibiotic resistant is a serious growing problem in contemporary medicine. This drives urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action [27]. Search of novel antimicrobial agents from plant sources is generally ensured by in vitro antimicrobial sensitivity test using agar well diffusion method.

In present study, different solventextract of *P. nilgiriensis* fruit and root showed a wide spectrum, being active against all of the micro-organisms tested. *K. pnemoniae*, which appears to be the most sensitive of the nine micro-organisms tested. The fungus *A. fumigates* is appearing to be the least sensitive species in this study. The data reported indicate that a high percentage of the plant extracts have antimicrobial activity, mainly against bacteria. The phenolic compounds, and in particular the methoxy phenols are responsible for the antimicrobial and antioxidant effects in smoked foods [28]. Water extract of fruit and root didn't show any significant activity against the used microbes. The non-activity of the water extract investigated in this study is in agreement with previous works which show that aqueous extracts of plant generally showed little or no antibacterial activities. Jayasinghe *et al.* [29] has been reported, the extracts of *Psychotria* sp. showed significant antimicrobial activity against *E. coli*, *B. subtilis* and *A. niger*. Results of the present study also evidently reveal the antimicrobial activity of *Psychotria* extracts against *Bacillus* and *Aspergillus* sp.

Khan *et al.* [30] reported the methanol extracts of leaves, root, stem and barks of *Psychotria microlabastra* showed broad spectrum of antibacterial activity against *B. subtilis*, *S. aureus*, *S. pneumoniae*, *E.coli*, *K. pnemoniae* and *P. aeruginosa*. Extract of the plant *P. microlabastra* didn't showed activity against moulds. The extracts of *P. nilgiriensis* fruit and root also showed significant activity against tested bacterial strains but it showed meager activity against the fungus. Giang *et al.* [31] had been reported polar fractions of *Psychotria reevesii* Wall. had higher level of tannin content so it is responsible for its antibacterial activity. Fractions of *P. reevesii* showed significant inhibitory activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Acetone extract of *P. nilgiriensis* fruit and root also showed higher amount of tannin content and tremendous inhibitory activity against *S. aureus* and *P. aeruginosa.*

CONCLUSION

As evident from the above discussion, acetone extract of *P. nilgiriensis* fruit and root contains notable chemical compounds that are responsible for its antioxidant activity. Acetone extract of fruit has significant antimicrobial activity against tested pathogens. This would suggest the use of the plant for the treatment of disorders related to oxidative stress and microbes. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

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