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ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT, ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF *PERSEA ODORATISSIMA* (NEES)

Pritee Pant*[1], Kapil Khulbe[2] and Charu Chandra Pant[3]

[1,2]Department of Botany, D.S.B. Campus, Kumaun University Nainital-263002, Uttarakhand, India.
[3]Department of Chemistry, Uttarakhand Open University Haldwani, Nainital, Uttrakhand, India.

***Corresponding Author: Pritee Pant**
Department of Botany, D.S.B. Campus, Kumaun University Nainital-263002, Uttarakhand, India.

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ABSTRACT
Present study deals with the phytochemical analysis, antioxidant and antibacterial activity of leaf extract of *Persea odoratissima*. Beside these GC-MS analysis of volatile oil of leaf was also done. *P. odoratissima* belongs to the family lauraceae used in the folk medicine as antiseptic and anti-inflammatory remedies. The two solvent methanol and hexane used for the extraction of leaves and different methods used for the exploration of the qualitative phytochemical analysis, antioxidant like total phenolic contain, total flavonoid contain, DPPH, Ferric reducing analysis, and antibacterial test by disc diffusion method. The oil sample was analyzed by GC and GC-MS and the components were identified on the basis of their RI values. Quantitative evaluations of methanol and hexane extract revealed that the presence of effective amount of phenol (88.06mgGAE/g dry weight and 0.64mgGAE/g dry weight respectively) and flavonoid (6.28mgQE/g dry weight and3.08 mgQE/g dry weight) Ferric reducing power of the plant found the 65.63 and 0.84AAE/g dry weight for methanol and hexane extract respectively. Plant showed high antioxidant potential by DPPH method (IC50 7.90µg/ml µg/ml and 118.84µg/ml for methanol and hexane respectively), which was showed close relation with ascorbic acid (IC50 5.48) used as a standard. The extract were tested against various bacterial strain at different concentration the inhibition zones were in the range of 15.38 mm to 6.5 mm in 100 mg/ml to 12.5mg/ml concentration respectively. GC-MS analysis of the leaf oil was found to be rich in Oxigenated sesquiterpenes (50.57%).

KEYWORDS: Antioxidant activity, phytochemical analysis, antibacterial activity, DPPH, GC-MS analysis.

1. INTRODUCTION
Several endogenous and exogenous causes like metabolism, chemicals and ionizing radiation leads to the formation of free radical, which are the main cause of oxidation of cells and tissue and induce many diseases. In addition when harmful forms of microbes multiply inside the body caused severe infections. To overcome these problems there is necessarily to introduce new substances of antioxidant and antimicrobial power.

Synthetic drugs are not only expensive and insufficient for the treatment of diseases but also often associated with many side effects. Therefore, there is also need to search new oxidation and infection fighting strategies.

The significance of the plant extracts are due to the occurrence of various phytochemicals such as phenolic acids, tannins, flavonoids, lignin, alkaloids, terpenoids, glycoside, quinones and other secondary metabolites. Studies have demonstrated that many of these phytochemicals contribute as antioxidants and antibacterial agent and systematic screening of the plant extract and phytochemicals result in the discovery of novel effective compounds. (Akinpelu and Onakoya, 2006).

There are many advantages of using medicinal plants, such as fewer side effects, quite less costly, better patient easiness, recognition due to long history of use and being renewable in nature. For this region researcher rejuvenated interest in the fields of phytochemistry, phytopharmacology, phytomedicine and phytotherapy (Benkeblia, 2004).

Out of 250 000 species of angiosperms and gymnosperms on the Earth surface, only around 15% have been phytochemically screened and 6% analyzed for biological activity (Fabricant, 2001, Verpoorte, 2000). Thus it is important to characterize different types of medicinal plants for their antioxidant, antimicrobial potential together with their phytochemical analysis.

With the arising worldwide interest in traditional systems of medicine and exploiting their potential based on different health care systems, the exploration of new drugs from the rich heritage of traditional medicine is

necessary. In this regard, one such significant plant is *Persea odoratissima* a member of Lauraceae family, an evergreen tree with rough and grey bark, dark green leaves and yellowish white coloured flower. The plant mostly found in sub-tropical and lower part of Himalayas at altitude of 2500 - 7000ft. The tree bark is used in the traditional medicine as anti-inflammatory and antiseptic remedies. The leaves are used to treat snake bite and burn wounds (Nguyen, et al, 1995).

A number of plants in the genus *Persea* are sources of secondary metabolites with exciting chemical structures and important bioactivities. Flavonoids, butanolides, lignans, alkaloids and sesquiterpenes have been reported from numerous species. The known chemical constituents of *Persea* are around 140 which include butanolides, flavonoids, lignans, sesquiterpenes, alkaloids, diterpenes and others (Jie Zhao, 2011).

Nature has blessed on mankind a very rich botanical wealth which serves to cure specific ailments and has been in vogue since ancient times around the world. The long term use of herbs in medicine is a sure indication of their value and usefulness and even world health organization records these as best source for the production of future herbal drugs.

The present investigation aimed at preliminary phytochemical analysis, antioxidant and antibacterial activity of leaf extract of the plant, as well as GC-MS characterization of its essential oil.

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Chemicals

Methanol, hexane, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, ascorbic acid, gallic acid, aluminum chloride, potassium acetate, sodium acetate, ascorbic acid, phosphate buffer, nutrient broth, dimethyl sulfoxide (DMSO), Muller Hilton agar, brain heart infusion agar, brain heart infusion broth were obtained from Himedia Laboratories Pvt. Ltd, Mumbai, India. Folin-Ciocalteu's reagent, Molisch's reagent, conc. H_2SO_4, Fehling's reagent, glacial acetic acid, conc. HCl, NH_4OH, Meyer's reagent, hexane, methanol were obtained from Merck, Mumbai, India. All chemicals used were of analytical grade.

2.1.2 Plant material

Fresh leaves of *P. odoratissima* were collected from hill state of Indian central Himalaya (Uttarakhand latitude and longitude) in the month of February 2016. The taxonomic identities of this plant were confirmed and a voucher specimen was deposited taxonomic laboratory at the Department of Botany, D.S.B Campus, Kumaun University, Nainital.

2.1.3 Bacterial strains

The bacterial strains, *Pseudomonas aeroginosa* MTCC-3542, *Proteus mirabilis* MTCC- 3310, *Klebisella Pneumonia* MTCC-7028, *Listeria monocytogenes* MTCC-657 were obtained from MTCC, Chandigarh, India.

2.2 Methods

2.2.1 Preparation of extract

P. odoratissima leaves were air dried at room temperature and ground in a pestle mortar. 10g of plant powder was extracted in 60 ml of methanol and hexane, filtered through Whatman filter paper No 1. and concentrated to dryness under reduced pressure and controlled temperature (40-50°C). The dried leaf extracts was dissolved in methanol and 90% dimethyle sulphoxide (DMSO) with hexane and stock solutions (1.0 mg/ml) were prepared for further analysis.

2.2.2 Extraction of volatile oil for GC, GC-MS

The essential oils were obtained from 750 g of fresh leaves of *P. odoratissima* by water-distillation using a Clevenger-type system for 12 h. The pooled organic phases were dried with sodium sulphate, filtered and the solvent was evaporated until dryness. Dried samples were stored at −25 ◦C in sealed glass vials.

2.2.3 Identification of components

Oil components were identified on the source of their retention indices RI and by assessment of their mass spectral fragmentation patterns with the respective literature (Adams, 2007).

2.2.4 Qualitative phytochemical screening

Phytochemical screening for the detection of the presence of different phytochemical classes was carried out by following standard procedures (Harbone, 1998).

2.2.5 Total phenolic and flavonoid content

Total phenolic and flavonoid content were determined by quantitative analysis of leaf extract performed by spectrophotometrically.

2.2.5.1 Total phenolic content

Total phenolics were estimated by following folin-ciocalteu's phenol reagent method of Singleton and Rossi (1965) with little modification. A standard curve of gallic acid was plotted using different concentrations of gallic acid (10-100µg/ml). Each sample (0.5ml) was mixed with 0.2 ml of Folin-Ciocalteu's reagent and 0.5ml of Na_2CO_3 (20%). After 30 min of incubation at room temperature absorbance was recorded at 765 nm against blank which contained only solvents. The concentration of total phenolic content was calculated as mg gallic acid equivalent/g dry weight, from the calibration curve of standard solution of gallic acid ($Y=0.003x$; $R^2 = 0.995$).

2.2.5.2 Total flavonoid content

Total flavonoid in the examined plant leaf extract was determined using spectrophotometric method (Willet, 2002) with some modification with quercetin as standard. Different concentrations (10-100 µg/ml) of

quercetin and test samples were prepared in water. Each sample (0.5ml) was mixed with 0.1 ml $AlCl_3$(10%), 0.1 ml of 1 M Potassium acetate, raising the final volume up to 5.0 ml by adding distilled water. After 30 min incubation at room temperature the absorbance were recorded at 415 nm. Based on the calibration curve of quercetin (Y= 0.006x; R^2=0.983) the flavonoid content of extract were calculated and expressed in terms of mg quercetin equivalents/ g of dry weight.

2.2.6 Frap (Ferric reducing antioxidant power) method

Frap assay was performed according to the methods of Benzie and Strain (1999) with some modifications. An amount of 100 µl extracted samples were mixed with 1.5 ml FRAP reagent in a test tube. Both samples and blank were incubated for 10 min at 37ºC and absorbance of the sample was determined against blank at 593nm. Different concentrations of ascorbic acid ranging from 10-100 µg/ ml were prepared for standard curve plotting.

The values obtained were expressed as mg of ascorbic acid equivalent per gram of dried extract, based on the calibration curve of ascorbic acid (Y= 0.022x; R^2=0.992).

2.2.7 Determination of antioxidant activity (DPPH Methods)

The antioxidant activity of the methanol extract of *P. odoratissima* was assessed on the basis of their scavenging effect of the DPPH (2, 2 -Diphenyl-1-picryl-hydrazyl) free radicals. DPPH radical scavenging ability was evaluated based on the standard method (Xie, *et al*., 2010) with some modifications. A fresh solution of DPPH (0.1m mole) was prepared in methanol and 2.0 ml of this solution was mixed with 2.0 ml of the extract at various concentrations (10-100 µg/ ml). The mixture of 2.0 ml DPPH in 2.0 ml methanol was used as control. After incubation of reaction mixture in dark for 30 min it analyzed spectrophotometrically at 517 against blank. Ascorbic acid was used as positive control. The free radical scavenging potential (%) of the leaf extract was calculated using the following formula:

Percentage of inhibition = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] × 100.

2.2.8 Antibacterial activity

2.2.8.1 Preparation of bacterial culture

Nutrient agar was used for the culturing the bacterial strain and incubated at 37 ±1ºC for 24 h and repeatedly sub-cultured in order to obtain pure isolation. Overnight broth culture of the respective bacteria strains were adjusted to turbidity equivalent to 0.5 McFarland standards (0.1 ml culture of the organisms was dispensed into 50 ml sterile nutrient broth and incubated for 24 h and standardized at 1.5 x 106 CFU/ml by adjusting the optical density to 0.1 at 600nm UVspectrophotometry (Tereschuk *et al*., 1997).

2.2.8.2 Antibacterial assay

Agar disc diffusion method (Bauer et al., 1966) was used to determine antibacterial activity. The dried extract was reconstituted with Dimethyl sulfoxide (DMSO) and serially diluted to achieve a stock solution of 100 mg/ml, 50 mg/ml, and 25 mg/ml, 12.5 mg/ ml were prepared.

Mueller hilton agar and brain heart infusion agar (for *Listeria monocytogenes*) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. Sterile discs of 6mm diameter cut from whatman filter paper were impregnated with 30µl of the solution of crude extracts prepared using DMSO solvent. The discs were evaporated. DMSO was used as negative and standard antibiotics (gentamycine and ampicillin were used as positive control. The plates were incubated at 37±1ºC for 24hr.

The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf extract. All experiments were conducted in triplicate and the results obtained were expressed as mean ± standard error (SE).

3. RESULTS AND DISCUSSION

Many members of lauraceae, have been used in traditional medicines, and thus interest many workers to investigate them they have been investigated chemically and biologically (Kumar *et al.,* 2017, Ahmad 2012, Showkat *et al*., 2004, Smith *et al*., 2002), and a few records have also been found on *P.odoratissima* during literature search.

Subedi, *et al*., 2011 reported its antibacterial and DPPH scavenging activity of methanol extract of its bark from Nepal. Bioactivity of leaves essential oil and trepinoid diversity has been carried out by Joshi *et al*, 2009, 2010; and Giang *et al*, 2006, 2011 reported that Chemical profile of the *n*-hexane-soluble fraction of the methanol extract of the bark.

3.1 Extract yield

The yield in methanol, and hexane extract were 6.5% and 0.5% respectively. Differences in yield of extract from different solvent might be attributed to the availability of extractable component of different polarities, and also signifies the importance of polar solvent as better extractants.

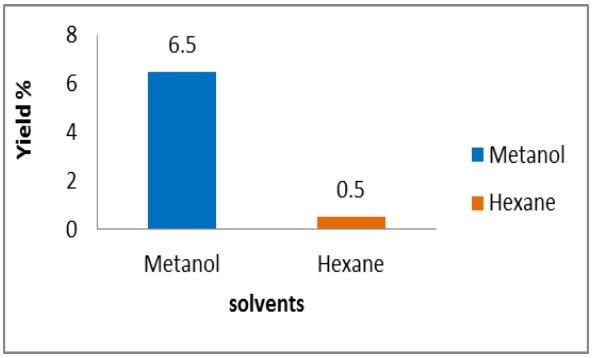


Fig. 1: Yield of extract in solvents.

3.2 Chemical Composition of the Essential oil of P.odoratissima

The oil sample was analyzed by GC and GC-MS and the components were identified on the basis of their RI values, co-injection with the available authentic samples and by comparison of their mass spectra with those reported in the literature (Adams, 2007).

The Gas Chromatographic examination of the oil showed the presence of more than sixty five peak, out of these forty seven components were identified which represented 95.46% of the total oil. The unidentified part of the oil was made up of several negligible constituents. The oil was found to be rich in Oxigenated sesquiterpenes (50.57%) while Sesquiterpene hydrocarbon (22.75%) of the oil. The Oxygenated monoterpeness (8.38%) and monoterpene hydrocarbons (13.76%). The major components of the oil were identified to be Geranyl valerate (14.77%) Bisabolol-n (8.66%), Rosifoliol (7.43%), Germacrene-D (6.23%) and Undecane (4.86%).

Many compounds have definite role in many alignments such as Bisabolol shows antioxidant, anticancer, anti-inflammatory anticholinestene activity (Kadir *et al*, 1991, Baylac 2003, van zyl 2006). Rosifoliol exhibited a significant, protective effect against linoleic acid oxidation (Rosa 2007). Germacrene D has been reported to have insecticidal activity against mosquitoes (Kiran 2007), as well as repellent activity against aphid (Bruce *et al*, 2005 and ticks (Birkett *et al*, 2008).

On the other hand, we could not get any traces of α-pinene, caryophyllene, valencene, cadinene which have been reported as major compounds by Joshi *et al*., 2010. This divergence in the chemical profile of *P. odoratissima* of collected sample can be attributed to either geo- graphical and ecological factors, or be an indication of the existence of different chemotypes of this plant, which can be a matter of interest for further work.

Table 1: Terpenoid composition of *P.odoratissima* leaf.

Compounds	Percentage in the Oil	RI value
Heptanone-2-methyl -4	0.85	901
4-methyl pentanoic acid	0.89	929
Heptene-1-ol-2E	0.72	956
Sabinene	0.61	968
Decane-n	0.96	999
Terpinene-α	0.70	1014
Methylheptanane	0.86	1020
Terpinene-γ	0.92	1044
Pentyl isobutanoate	0.87	1052
Mentha-2,4-diene	0.89	1055
Isopentyl butanoate	0.18	1058
Sabinene hydrate-cis	0.14	1062
Sabinene-cis	1.20	1068
Undecane	**4.86**	**1099**
Pinocamphone-trans	0.59	1263
Verbenyl acetate	0.43	1267
Decen-1-ol	0.60	1269
Pentylalyl buturate	1.61	1279
Tridecane-n	2.79	1299
Tetradecane-n	1.78	1399
α-copene	0.67	1474
Germacrene-D	**6.23**	**1516**
Bisabolene-E	4.81	1551
Curcumene	0.42	1578
Hexenyl benzoate	0.26	1595
Sesquithuriferol	3.63	1603
Cubenol-1-epi	2.06	1615

Sesquilavandulol-E	0.73	1621
β- Selinene	0.69	1648
Geranyl valerate	**14.77**	**1663**
Farnesal-2Z,6Z	0.16	1672
Bisabolol-n	**8.66**	**1678**
Heptadecane-n	3.56	1700
Sesquicineol-2-one	2.16	1705
Tridecenol acetate	3.17	1712
Bulnesol	1.68	1719
Rosifoliol	**7.43**	**1755**
Farnesol	0.73	1777
Bisabolol acetate	0.62	1784
Hexadecane	0.96	1797
Octadecane	1.15	1800
Isopropyl myristate	0.73	1823
Cryptomeridial	0.86	1940
Nonadecane	1.53	1999
Geranyl linalool	2.55	2027
Palmitic Acid	0.29	2040
Phytol	2.50	2218
Oxigenated monoterpenes 8.38 % **Monoterpene hydrocarbons 13.76%** **Oxigenated sesquiterpenes 50.57%** **Sesquiterpene hydrocarbons 22.75%**		

3.3 Qualitative phytochemical screening

Results revealed that leaves of *P. odoratissima* contain many phytochemical constituents which might be responsible for many pharmacological activities reported in folk medicine. Phytochemical screening showed the presence of glycoside, alkaloids, terpenoid, phenols flavonoids and volatile oils in both the extracts.

Out of total 13 tests performed, 10 tests were found positive in methanol extract and 6 tests were positive in hexane extract. Quinon, tannin, carbohydrate and protein which were present in methanol extract but not in hexane extract. Phalobatanin, resin and saponin were totally absent in both the solvents. Levels of secondary metabolites are both environmentally induced as well as genetically controlled (Makkar 2007).

In phytochemical screening, presence of various phytochemicals in both the extracts shown that the plant is rich in various medicinal components thus can be used as a potential health promoting source.

Glycosides improve cardiac output and reduce heart diseases like congestive heart failure and cardiac arrhythmia etc. (Doss *et al*, 2011). Similarly, flavonoids, tannin and phenols acts as primary antioxidant and possess antimicrobial, anti-inflammatory, antiviral, antiallergic, anticancer activities etc. (Yun et al., 2015, Ozçelik *et al*, 2011, Polterait 1997). Other important groups of compounds are Alkaloids, which exhibit many therapeutic effects like antimicrobial, sedatives; anti-inflammatory, antiallergic etc. (Stray1998, Okwu *et al*, 2004).

Table 2: Phytochemical analysis of *P.odoratissima* leaves extract.

Sr. no.	Phytochemicals	Methanol	Hexane
1.	Carbohydrates	+	_
2.	Proteins	+	_
3.	Alkaloids	+	+
4.	Glycosides	+	+
5.	Tannin	+	_
6.	Flavonoids	+	+
7.	Phenol	+	+
8.	Volatile oil	+	+
9.	Terpinoid	+	+
10.	Saponin	_	_
11.	Resin	_	_
12.	Phalobatanin	_	_
13.	Quinon	+	_

+ Present, - Absent.

3.4 Total phenolic and flavonoid compounds

Phenol and flavonoids show antioxidant activity (Espin *et al*., 2000, Halliwell, 2001, Duh *et al*., 1999) and their effects on human nutrition and health is considerable. Phenolics act as free radical terminators (Om Prakash *et al*., 2007) and in invitro system they are measured in terms of gallic acid equivalent. Total phenolic content of the methanol and hexane extract of *P. odoratissima* leaves is measeard 88.06mg GAE/ g dry weight, and 0.64mg GAE/ g dry weight respectively.

The mechanism of action of flavonoids are through scavenging or chelating process (Kessler *et al*., 2003), and in present report is expressed in terms of quesertine equivalent. Total flavonoid contain of the methanol extract of is 6.28mg QE/g dry weight, while in hexane extract it is found 3.08mg QE/g dry weight.

Free radicals are accountable for many diseases such as bacterial and parasitic infections, inflammation, reperfusion injury, lung damage, cardiovascular disorders, atherosclerosis, neoplastic diseases and aging (Thomas and Kalyanaraman, 1997). They are also concerned in autoimmune disorders like rheumatoid arthritis (Halliwell 1997; Beckman and Ames 1998). Past few years have witnessed growing interest in the search of new natural antioxidants because production of reactive oxygen species (ROS) and oxidative stress which is linked to many diseases and enormous use of synthetic antioxidants leads to troubles of toxicity. Therefore, identification of new source of natural antioxidants is more essential. The high value of phenolic and flavonoid content of different plant extracts used is a good indication of high potential of its antioxidant activity.

3.5 Frap assay

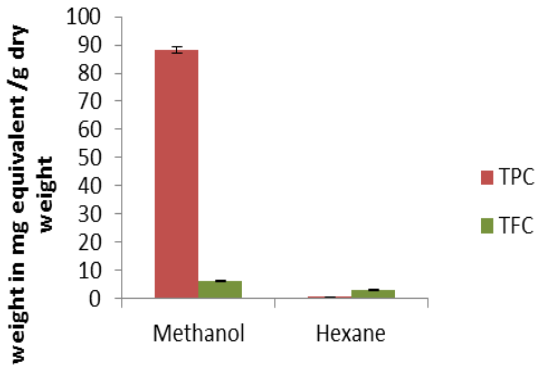
The ferric reducing capacity of a compound serves as a significant indicator of its potential antioxidant. This reducing capability depends on the presence of reductants (Duh *et al.*, 1999) which show antioxidative potential by donating a hydrogen atom thus break the formation free radical chains (Gordon, 1990). The presence of reducing agent in plant extract causes the reduction of the Fe3+ /ferricynide complex to the ferrous form, which is measured in terms of ascorbic acid equivalent.

P. odoratissima exhibited significant free radical scavenging ability with reducing ability of 65.63 mg and 0.84 mg dry weight for methanol and hexane extract respectively.

This suggests that plant bear therapeutic potential and could highly be considered as novel source for drug discovery.

Table 3: Total phenolic and flavonoid content and ferric reducing antioxidant power (mg /g dry weight) in *P. odoratissima* leaf extract.

Constituents	Methanol extract	Hexane extract
Total phenolic content mg GAE/ g dry weight	88.06±1.09	0.64±0.05
Total flavonoid content mg QE / g dry weight	6.28±0.24	3.08±0.04
Ferric reducing antioxidant power assay mg AAE/g dry weight	65.63±0.47	0.84±0.02



TPC- Total phenolic content (mg GAE/g dry weight)
TFC-Total flavonoid content(mg QE/g dry weight)
Fig. 2: Total phenolic and flavonoid content of leaf extract.

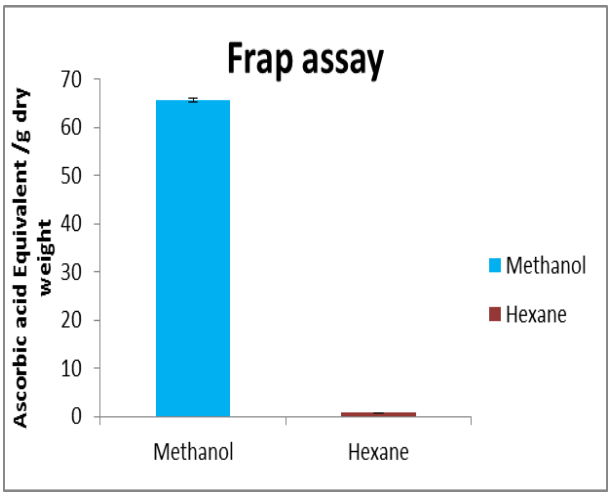


Fig. 3: Ferric reducing antioxidant power (Frap) assay.

3.6 Free radical scavenging activity

DPPH is a free radical and produces deep purple colour in methanol solution. The principle of this assay is based on the formation of yellow coloured diphenyl-picryl from reduction of purple coloured methanolic solution of DPPH in the presence of hydrogen donating antioxidants substance.

Present study reveals a concentration dependent inhibition of DPPH by the methanol (63 to 96%) and hexane (25 to 42%) with an IC_{50} as 7.90µg/ml and 118.84 µg/ml respectively.

Interestingly, the IC_{50} value of methanol extract comparable with ascorbic acid (5.48 µg/ml) indicates the high free radical scavenging property which may be either due to transfer of hydrogen atom or by transfer of an electron.

Further, positive correlation observed between antioxidant activity and phenolic and flavonoid content, is might be due to the scavenging activity of diverse compounds such as flavonoids, phenols and other phytochemicals acting singly or synergistically. The high antioxidant activity of polar extract (methanol) in the present investigation supports a linear correlation between total phenolic and flavonoid content and their antioxidant capacity. These findings are similar with respect to antioxidant potential of plant extract, which we argued that these could be possible synergism between phenolic compounds and their phytochemical released in the polar solvents.

Table 4 – % of inhibition and IC_{50} value of two extracts of plants and standard.

Concentration	Ascorbic acid	IC_{50} (µg/ml)	Methanol Extract	IC_{50} (µg/ml)	Hexane Extract	IC_{50} (µg/ml)
10	91.14 ±0.97	5.48±0.5	63.17± 0.5	7.90±0.06	21.55±0.59	118.84±1.13
30	92.44±0.19		72.41±0.47		28.57±0.77	
50	93.88±0.58		91.36±0.32		32.98±0.75	
70	94.92±0.30		94.98±0.16		36.36±0.73	
90	97.26±0.30		95.01±0.95		40.25±0.74	
100	98.17±0.44		96.51±0.23		42.07±0.30	

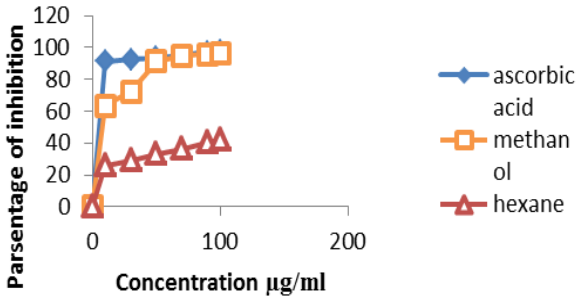


Fig. 4: Percent Inhibition of DPPH by plant extracts and standard.

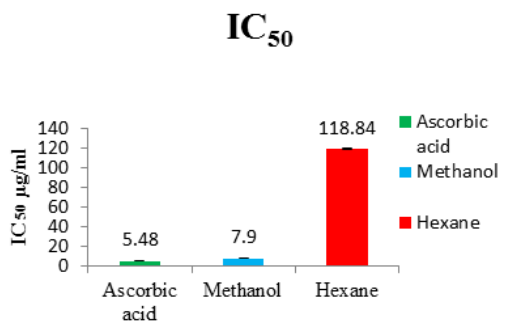


Fig. 5: IC_{50} value of leaf extract of *P.odoratissima* and standard.

3.7 Antibacterial activity

With the appearance of multi-drug resistance in pathogenic bacteria as well as adverse side effects of certain antibiotics has arisen enormous interest in the search for new antibacterial drugs of plant origin.

Plants are crucial source for the development of new chemotherapeutic agents. Many reports are available on the antiviral, antifungal, antihelmintic, antimolluscal, antibacterial and anti-inflammatory properties of plants. Some of these explanations have helped in identifying the active principle responsible for such actions and in the developing drugs for the remedial use in human beings.

Antibacterial activity of the leave extract conducted at four concentration of extract (100, 50, 25, 12.5 mg/ml) prepared by serial dilution method. Methanol extract has shown highest zone of inhibition against *Proteus mirabilis* followed by *P. aeriginousa* and *K. pnemoniae*. Antibacterial testing of hexane extract indicated significant activity against gram positive bacteria *L. monocytogenes* while the methanol extract not active against this bacterium.

Previously Joshi *et al*, 2010 reported the essential oil of *P. odoratissima* active against *E.coli, salmonella enteric,* and *staphylococcus aureus* Subedi *et al.*, 2011 also investigated antibacterial activity of methanol extract of *P.odoratissima* against *staphylococcs aureus*. No bioactivity of the plant has previously been performed against *Klebisella pnemoniae, Proteus mirabilis, Listeria monocytogenes*

These results revealed that antibacterial activity of the extract enhanced by increasing the concentration of the extract.

Subedi *et al.,* 2011 reported that plant extract did not show activity against *Pseudomonas aeriginousa* but the results of this study have shown effective inhibitory activity against this pathogen.

The medicinal plants studied appear to have a broad spectrum antimicrobial activity, they could be useful in antiseptic and disinfectant formulations as well as in chemotherapy. The effectiveness of a medicinal plant may not be due to one main active constituent, but to the mutual action of various compounds originally in the plant (Gonzalez *et al*., 1994) including essential oils, flavonoids and triterpenoids and other compounds of phenolic nature or free hydroxyl group, which are classified as active antimicrobial compounds.

Since *P.odoratissima* showed effective amount of phenolic compounds which are also recognized to have antibacterial activities. High concentration of phenolic compound acts as a protoplasmic poison by penetrating and disrupting bacterial cell wall in addition to precipitating the cell proteins (O'Connor and Rubino, 1991). In lower concentrations, it inactivates the cellular enzyme system causing leakage of essential metabolites from the cell (Widmer and Frei, 2003).

Table -5: Antibacterial activity of leaf extract in different concentration.

			Zone of inhibition(mm)								
Sr No.	Bacteria	Gen 10 mcg	Amp 10 mcg	Methanol extract				Hexane extract			
				100mg	50mg	25mg	12.5mg	100mg	50mg	25mg	12.5mg
1.	*Pseudomonas aeruginosa* MTCC 3542	29±2.08	20.3±0.88	13±0.57	12±0.57	11±0.65	8.6±0.65	8.6±0.33	8.10±.16	7.3±0.33	7±0.28
2.	*Klebsiella pnemoniae* MTCC7028	30±1.1	15.33±2.6	13±0.57	11.6±0.88	11±0.57	9±0.57	8.5±0.2	7.8±0.08	6.5±0.14	NA
3.	*Proteus mirabilis* MTCC 3310	31±2.08	15.6±2.3	15.3±0.8	15±0.57	13±0.57	11±0.57	NA	NA	NA	NA
4.	*Listeria monocytogenes* MTCC 657 Gram (+)	26±2.08	14±0.57	NA	NA	NA	NA	13.5±0.28	12±0.57	9.5±0.29	8.1±0.44

NA= Not Active.

4. CONCLUSION

To find out the real value of medicinal flora the scientific study should be essential. Present study revealed that this plant is a valuable reservoir of natural antioxidant and antibacterial compounds due to the combined action of various types of phytochemicals and very effective amount of phenolic compounds in methanol extract act as a potent source of antimicrobial and antioxidant agent. This study leads to their use as safe alternatives to synthetic antioxidant and antimicrobial drugs.

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