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Qualitative and Quantitative Phytochemical Analysis and Antioxidant Activity of *Pancratium zeylanicum L.* Bulb – An Endemic Medicinal Plant

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ABSTRACT

Pancratium zeylanicum L. belonging to Amaryllidaceae, an endemic plant of Indian sub-continent. The present works was designed to identifying the bioactive compounds and determines the *in-vitro* antioxidant activity of the ethanol and methanol extracts of *Pancratium zeylanicum* bulb. Phytochemical analysis was performed using qualitative and quantitative phytochemical screening with standard procedures. FTIR with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode with a resolution of 4 cm^{-1}. In GC-MS the interpretation of mass spectrum was conducted using database of National Institute Standard and Technology (NIST), library 2008. The antioxidant activity was evaluated by DPPH (Diphenylpicryl Hydrazyl radical scavenging method), nitric oxide, hydrogen peroxide, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical cation) radical scavenging, and FRAP (Ferric reducing ability of plasma) assays. The result reveals that some of the phytochemicals constituents such as alkaloids, flavonoids, tannins, steroids and triterpenoids were present in both the extracts; whereas glycosides and fixed oils are present in PZBM extract, and saponins and gum and mucilage were present in PZBE extract. The anthraquinone is absent in both the extracts. The total phenol, flavonoids and alkaloids presents in ethanol and methanol extracts is 25.67±1.43 mg/g and 29.41±1.80 mg/g, 27.20±1.15 mg/g and 31.26±0.39 mg/g, 24.16±0.38 mg/g and 26.52±0.67 mg/g. In FTIR analysis the ethanol and methanol extracts of *P. Zeylanicum* bulb have active functional groups like secondary amine, alkene, aldehyde, alkyl aryl ether, ester, aliphatic ether, halo compound, etc. GCMS analysis of ethanol and methanol extracts showed the presence of 90 and 90 components with 22 and 20 known bioactive compounds respectively. The antioxidant activity was found to be increased with increasing concentration of ethanol and methanol extracts. The IC_{50} value of DPPH, Nitric oxide, Hydrogen peroxide, $ABTS^{+}$ and FRAP in ethanol extract is 163.92±1.88, 349.37±1.2, 147.64±2.19, 98.65±1.29, 194.22±1.74 and in methanol extract is 147.29±2.46, 194.84±0.78, 228.74±0.99, 206.43±1.55, 117.39±2.61 respectively. Ethanol and methanol extracts of *P. zeylanicum* bulb is a potential source of natural antioxidants and serves as an effective free radical scavenger and inhibitor. Hence, *P. Zeylanicum* might be a good plant-based pharmaceutical product for several diseases caused by free radicals.

***Key words*:** *Pancratium zeylanicum,* FTIR, GCMS, DPPH radical scavenging, Ethanol, Methanol extracts

Plants have been used as medicines for thousands of years around the world for the prevention and treatment of different diseases, as well as a natural source of therapeutically active compounds [1]. Compared to modern synthetic drugs, herbal remedies from medicinal plants are more compatible, effective, cheaper, and safer to the human body [2]. To overcome the lack of coverage provided by the conventional health care system, rural communities use herbal medicines for primary health care. In the whole World approximately 90% of people in rural areas depend on the medicinal plants to meet primary health needs [3]. Compared to synthesized medicines, natural products offer a safer alternative. Only 15% of the known sources in the World have been screened for their therapeutic purpose.

A variety of infections can be cured using herbal medicines, due to their bioactive molecules. Compared to commercial antibiotics, herbal products are many times more effective with minimal side effects. Spices play a crucial role in enhancing the taste and aroma of food. It is also finding its place in traditional folklore medicine which is used to reduce the pain, gives protection to cells from damage, helps in detoxification

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[4]. Phytochemicals in plant materials are biologically active compounds that play a crucial role in the body's metabolic process. Biologically active components are abundant in plants [5]. Flavonoids, tannins and phenols are natural antioxidants that help keep the body healthy.

Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species in order to survive. Natural antioxidants have drawn a lot of attention in recent years because of their potential health benefits [6-7]. Researchers have been screening medicinal and food plants for antioxidant properties for the past few decades in an effort to find effective remedies for a variety of diseases [8]. Multiple diseases are caused by oxidative stress, including atherosclerosis, arthritis, cardiovascular disorders, Alzheimer's disease, and cancer [9]. It is necessary to replace synthetic antioxidants with natural antioxidants since synthetic antioxidants can have many side effects. Phenolic compounds are among the most important secondary metabolites in numerous medicinal plants. In human life, phenolic compounds have beneficial effects due to their antioxidant activity, which is largely due to their redox properties, which allow them to act as reducing agents, hydrogen donors and radical scavengers [10].

The Amaryllidaceae family has attracted attention as a source of valuable biologically active alkaloids. There are about 15 species of *Pancratium* in the Mediterranean, Africa, and Asia [11]. Amaryllidaceae contain a wide range of alkaloids that exhibit antitumor, antiviral, antibacterial, antifungal, antimalarial, analgesic, and cytotoxic activities [12]. Bulbous plants have been shown to contain unique, biologically active compounds that could be used to develop pharmaceutical products. Among Amaryllidaceae, there are numerous bioactive compounds with different structures that are specific to the species. *Pancratium zeylanicum* A herbaceous, bulbous plant, used as a substitute and adulterant of Indian Squill, a drug used as a cardiotonic, diuretic, and expectorant, prescribed also for bronchial troubles. Only few reports are available for antioxidant and anticancer activity of *P. zeylanicum* [13].

In this study the chemical compositions, FTIR, GCMS and antioxidant activity (DPPH radical scavenging activity, ABTS radical scavenging activity, nitric oxide, hydrogen peroxide and FRAP) of *P. zeylanicum* bulb ethanol and methanol extracts were reported. To our best knowledge, there is no research on the phytochemical compounds, FTIR, GCMS activities of *P. zeylanicum* bulb.

MATERIALS AND METHODS

Preparation of plant extract

P. zeylanicum dried bulbs (100 grams) were subjected to Soxhlet extraction using the adjusted methodology of Elwekeel *et al*. 2013. Ethanol and methanol solvents were used for this extraction. After extraction, the solvent was evaporated by vacuum solvent evaporator. Then, the extracts were stored at 4°C for investigation of phytochemical analysis and pharmacological studies.

Preliminary phytochemical screening

Presence of bioactive phytochemicals like alkaloids, anthraquinones, glycosides, saponins, phenols, tannins, flavonoids, steroids, terpenoids, fixed oils and gum and mucilages in *P. zeylanicum* bulb ethanol (PZBE) and *P. zeylanicum* bulb methanol (PZBM) extract was carried out by standard methodologies.

Quantification methods

Quantification of alkaloids

The PZBE and PZBM extract were dissolved in 0.5 mL of 0.1 m mol/L HCl and 2 mL deionized water. After the solution was transferred to a 25 mL volumetric flask, water was added to the flask scale and then filtered. Following that, 2 mL of deionized water and 2 mL of bromocresol green were transferred to a dividing funnel and shaken, and then 10 mL of $CHCl_3$was added and vigorously shaken for 2 minutes. The solution layer was collected and measured absorbance at 411 nm.

Quantification of total phenolics

In this method 150 µL of PZBE and PZBM extracts were taken into a series of test tubes and made up to 1 mL with distilled water. The blank was a test tube containing 1 mL of distilled water. All the test tubes, including the blank, were then added 500 µL Folin-Ciocalteau Phenol reagent (1 N). All test tubes were added 2.5 mL of sodium carbonate solution (20%) after five minutes. Vortexing was performed to mix the contents well and incubation was performed for 40 minutes in the dark. The presence of phenolics was indicated by the blue colour formed on the test tubes during incubation. Following incubation, the absorbance was measured at 725 nm against a reagent blank. A gallic acid standard was prepared and the results were expressed as Gallic acid equivalents (GAE). The analyses were performed in triplicates.

Quantification of total flavonoids

About 800 µL of PZBE and PZBM extracts were taken in different test tubes and 2 mL of distilled water was added to each test tube. The blank was a test tube containing 2.5 mL of distilled water. All test tubes were then treated with 150 µL of 5% $NaNO_2$, which was then incubated at room temperature for six minutes. In all the test tubes, including the blank, 150 µL of 10% $AlCl_3$ was added after incubation. Incubation at room temperature for 6 minutes was performed on all test tubes. Then 2 mL of 4% NaOH was added to all the test tubes which were then made up to 5 mL using distilled water. All test tubes were vortexed well and allowed to stand at room temperature for 15 minutes. A spectrophotometric measurement at 510 nm revealed that the pink color developed as a result of flavonoids. The flavonoids were measured using rutin as the standard. The experiments were conducted in triplicate, and the results were expressed in rutin equivalents (RE).

FTIR analysis

The FTIR analysis of the PZBE and PZBM extracts were conducted using the potassium bromide (KBr) pellets (FTIR grade) using the Jasco FTIR-6300 Fourier transform infrared spectrometer equipped with a JASCO IRT-7000 Intron Infrared Microscope using transmittance mode with a resolution of 4 cm^{-1} (JASCO, Tokyo, Japan).

GCMS analysis

GCMS analysis was carried out to identify the phytochemical constituents of the PZBE and PZBM extracts. GCMS model, Perkin Elmer, Clarus 680 and Helium (1 mL/min) was used as a carrier gas. 1 µL of each extract was injected into the instrument and the injector temperature was set at 260°C during the chromatographic run. The mass detector conditions were transfer line temperature 240°C, ion source temperature 240°C and ionization mode electron impact at 70 eV, a scan time 0.2 second and scan interval of 0.1 second. The oven temperature was fixed in starting at 60°C for 2 min then the temperature was changed increased into 300 °C at the rate of 10 °C min^{-1} and this temperature was held for 6 min. The GCMS analysis was performed in Sophisticated Instrument

Facility (SIF), VIT University, Vellore and TurboMass ver 5.4.2 software was used. The interpretation of mass spectrum GCMS was conducted using database of National Institute Standard and Technology (NIST), library 2008.

Antioxidant assays

Diphenylpicryl hydrazyl (DPPH) radical scavenging activity

Antioxidant activity of PZBE and PZBM extracts was analyzed by Spectrophotometric method on the basis of determination of scavenging activity of DPPH free radical by [14]. DPPH, (100mM, 22 mg of DPPH) was accurately weighted and dissolved in 100 ml of methanol. From this stock solution, 10 ml was taken and diluted to 100 ml using methanol to obtained 100 mM DPPH solution. Microtitre plates containing 96 wells were used for the assay. In the microtitre plate, 10µl of each sample or standard solution were added separately to 200 µl of DPPH solution. The final concentration of the least and standard solution used are 1000 to1.953µg/ml. The plates were incubated at 37°C for 20 minutes and the absorbance of each well was measured at 490 nm, using ELISA reader against the corresponding test and standard blank and the remaining DPPH was calculated. IC_{50} (Inhibition Concentration) is the concentration of the sample required to scavenging 50% of DPPH free radicals.

$$\text{Percent of Inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide assay of PZBE and PZBM extracts were carried out by following the methoddescribed by Hazra [15]. The reaction mixture (6 ml) containing PZBM and PZBE extract (10 mm, 4 ml) and 1 ml of DMSO were incubated at 25ºC for 90 minutes. After incubation 0.5 ml of the extraction mixture containing nitrate was removed and 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 ml of NEDD was added, mixture and allowed to stand for 30 minutes in different light at room temperature. The absorbance of this solution was measured at 540 nm using ELISA reader against corresponding blank solution. IC_{50} value obtained is the concentration of the sample required to inhibition 50% NO radical.

Hydrogen peroxide radical scavenging activity

The ability of an extract of PZBE and PZBM extracts to scavenge hydrogen peroxide was tested according to the method of [16]. PBS (pH 7.4) was dissolved in 20 mM H_2O_2.Various concentrations of 1 ml of the extracts or standard in methanol were added to 2 ml of H_2O_2 solution in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without H_2O_2.

$ABTS^+$ radical scavenging activity

Various concentrations of the PZBE and PZBM extracts and standards (2ml), 1 ml of distilled DMSO and 0.16 ml of $ABTS^+$ solution were added to make a final volume of 1.36 ml. After 20 minutes at 734 nm, absorption was measured spectrophotometrically using an ELISA reader. Blank is maintained without $ABTS^+$. IC_{50} value obtained is the concentration of the sample required to inhibit 50% $ABTS^+$ radical mono cation.

FRAP radical scavenging activity

The total antioxidant potential of PZBE and PZBM extracts was determined using ferric reducing ability of plasma FRAP assay as a measure of antioxidant power by Benzie and Strain 1996. The FRAP reagent contains 25 ml of acetate buffer and 2.5 ml of ferric chloride solution. It was freshly prepared and warmed to 37°C before use. A mixture of 900 µl of FRAP reagent, 90 µl of water, and 30 µl of test sample was then prepared. The reaction mixture was then incubated at 37°C for 30 minutes and the absorbance was recorded at 593 nm. An intense blue coloured complex were formed when ferric tripyridyltriazine complex were reduced to ferrous form. The absorption at 540 nm was recorded. The calibration was plotted with absorbance at 593 nm Vs concentration of ferrous sulphate in the range 0.1 mM both aqueous and *P. zeylanicum* extract. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidants L-ascorbic acid.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

In this study preliminary phytochemical screening of PZBE and PZBM extracts was analyzed. The result reveals that some of the phytochemicals constituents such as alkaloids, flavonoids, tannins, steroids and triterpenoids were present in both the extracts; whereas glycosides and fixed oils are present in PZBM extract, and saponins and gum and mucilage were present in PZBE extract. The anthraquinone is absent in both the extracts (Table 1).

Table 1 Qualitative analysis of PZBM and PZBE extract + indicate present of secondary metabolites - indicate absent of secondary metabolites

Compounds	Tests	Ethanol	Methanol
Alkaloids	Dragendroff's test	+	+
	Mayer's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
Flavonoids	10% HCl & 5% NaOH test	+	+
	Alkaline test	+	+
Tannins	5% $FeCl_3$ test	+	+
Steroids	Libermann - Burchard's test	+	+
Triterpenoids	Libermann - Burchard's test	+	+
	Salkowski's test	+	+
Saponins	Foam test	+	-
Glycosides	Killer &Kilian test	-	+
Gum & Mucilages	Whistler & BeMiller test	+	-
Fixed oils	Spot test	-	+
Anthraquinones	NH_4OH test	-	-

Table 2 Quantitative analysis of PZBE and PZBM extracts

Quantification	Yield value in mg/g	
	Ethanol	Methanol
Alkaloids	24.16±0.38	26.52±0.67
Flavonoids	27.20±1.15	31.26±0.39
Phenolics	25.67±1.43	29.41±1.80

Quantitative analysis

According to data depicted in (Table 2), PZBE and PZBM extracts contain an appreciable level of secondary metabolites content. The total phenol, flavonoids and alkaloid contents of ethanol and methanol extracts was 25.67±1.43, 29.41±1.80, 27.20±1.15, 31.26±0.39 and 24.16±0.38, 26.52±0.67 mg/g. All the three secondary metabolites were highly isolated in methanol extract.

FTIR analysis

FTIR characterization studies are used to identify the functional groups of the phytochemicals present in the PZBE and PZBM extracts. Table 3and 4 shows 12 and 13 different intense peaks at wave numbers which are corresponds to functional groups of the ethanol and methanol extracts of *P. zeylanicum* bulb. The medium band was observed at 3327.17cm^{-1} corresponds to N-H stretching phenols and alcohols. The weak band at 1678.33cm^{-1} indicates the presence of C=C stretching alkene. A strong band was observed at 1194.26cm^{-1}corresponds C-O stretch alkyl aryl ether. The bands 1167.82, 1080.50, 810.24 and 687.73cm^{-1} are assigned to C-O stretching, C=C banding and C-Br stretching ester, aliphatic ether, alkene and halo compound in ethanol extract, respectively (Fig 2). Whereas in methanol extract the strong and broadband's shown at absorption peak 3378.19 and 2961.39cm^{-1} are designated to O-H stretching and C-N stretched alcohol and amine salt. The strong peaks were observed at 1491.44, 996.61, 698.68, 508.26 and 479.12cm^{-1} corresponds to N-O stretching, C=C banding, C-I stretching and C-Br stretching nitro compound, alkene, and halo compound respectively (Fig 3). Hence this result concluded that the ethanol and methanol extracts of *P. zeylanicum* bulb have active functional groups like secondary amine, alkene, aldehyde, alkyl aryl ether, ester, aliphatic ether, halo compound, etc. These functional groups are associated with the bioactive phytochemicals in the bulb extract.

Table 3 FTIR analysis of PZBE extract

Frequency range	Bond	Functional groups
3327.17(m)	N-H stretching	secondary amine
3020.96(m)	N-H stretching	secondary amine
2906.22(m)	C-H stretching	alkene
1678.33(w)	C=C stretching	alkene
1375.10(m)	C-H banding	aldehyde
1194.26(s)	C-O stretching	alkyl aryl ether
1167.82(s)	C-O stretching	ester
1080.50(s)	C-O stretching	aliphatic ether
936.21(s)	C=C banding	alkene
810.24(m)	C=C banding	alkene
720.19(s)	C=C banding	alkene
687.73(s)	C-Br stretching	halo compound

GCMS analysis

GCMS analysis of PZBE and PZBM extracts showed the presence of 90 and 90 compounds at the different retention time (Fig 4). In these 90 compounds, 22 bioactive compounds are identified in PZBE. GCMS analysis of methanol extract of *P. Zeylanicum* has 90 phytochemical constituents with 20 bioactive compounds. (Table 5-6) reported 22 and 20 bioactive compounds with molecular formula, molecular weight, CAS No and bioactive uses and retention time from 4.0 to 21.0 (Fig 5). The both extracts have the bioactive uses of antimicrobial, anti-inflammatory, cytotoxic properties, antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic, 5-apha reductase inhibitor, anti-angiogenic and anti-tumor, antifouling, antidiabetic, antiarthritic and herbicide properties (Table 5-6).

Antioxidant activity

DPPH radical scavenging activity

The PZBE and PZBM extracts exhibited free radical scavenging activity by inhibiting DPPH radical, which was dependent on concentrations of the extracts. The well-known antioxidant, standard ascorbic acid and rutin, showed high degree of free radical-scavenging activity than that of the plant extracts at each concentration points. The highest inhibition percentage (81.00±1.53) was observed in PZBM extract at 1000 µg/ml concentration, whereas PZBE shows less inhibition percentage **(**79.22±1.01**)** at the same concentration (Table 7). The IC_{50} of the crude ethanol extract of bulb was 163.92±1.88 µg/ml and methanol extract was 147.29±2.46 µg/ml, respectively, whereas IC_{50} value for the standard ascorbic acid was 109.35±0.48 µg/ml and rutin 108.22±1.47 µg/ml (Table 12).

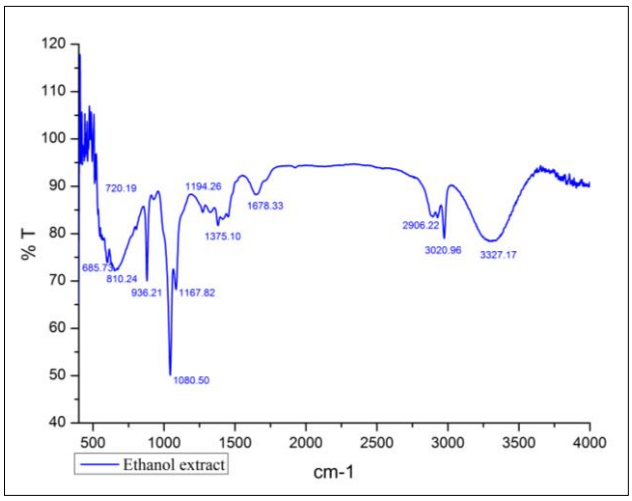

Fig 2 FTIR analysis of PZBE extract

Table 4 FTIR analysis of PZBM extract

Frequency range	Bond	Functional groups
3378.19(s,b)	O-H stretching	alcohol
2961.39(s,b)	N-H stretching	amine salt
1658.19(m)	C=C stretching	alkene
1491.44(s)	N-O stretching	nitro compound
1320.82(m)	O-H banding	alcohol
1024.44(m)	C-N stretching	amine
996.61(s)	C=C banding	alkene
963.74(s)	C=C banding	alkene
698.68(s)	C=C banding	alkene
621.07(m)	C=C banding	alkene
508.26(s)	C-I stretching	halo compound
486.29(s)	C-I stretching	halo compound

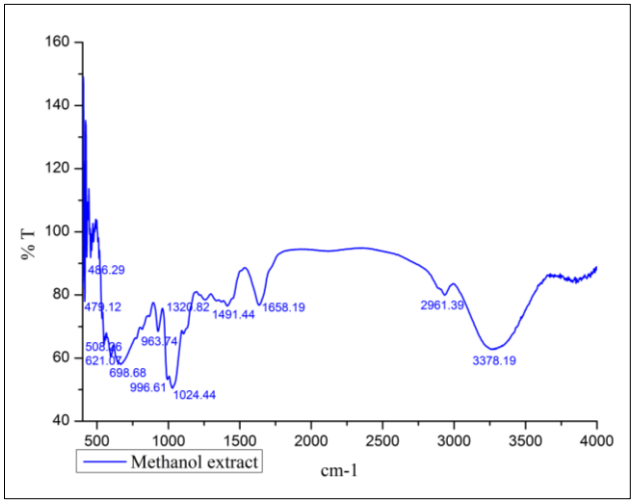

Fig 3 FTIR analysis of PZBM extract

Table 5 GCMS analysis of PZBE extract

Compound name	Molecular formula	Molecular weight	CAS No	Bioactive uses
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	028564-83-2	Antimicrobial, anti-inflammatory activities [17]
2- Furancarboxaldehyde, 5-(Hydroxymethyl)-	$C_6H_6O_3$	126.1100	67-47-0	Anti-carcinogenic [18]
1H-Pyrrole-2,5-dione	$C_4H_3NO_2$	97.0721	541-59-3	Antioxidant properties [19]
9-Eicosene, (E)-	$C_{20}H_{40}$	280	42448-90-8	Anti-microbial and cytotoxic properties [20]
1-Heptadecene	$C_{17}H_{34}$	238.4519	6765-39-5	Antifungal and antibiotic properties [21]
d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$	210.18	5328-64-3	Antioxidant and antibacterial activities [22]
Cyclotetradecane	$C_{14}H_{28}$	196.3721	295-17-0	Used in cosmetic and topical medicinal preparations where good absorption through the skin is desired [18]
Dibutyl phthalate	$C_{16}H_{22}O_4$	278.3435	84-74-2	Antioxidant, anticancer activities [23]
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	57-10-3	Anti-inflammatory, antibacterial, antioxidant, hypocholesterolemic nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic, 5-Alphareductase inhibitor [24]
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455	60-33-3	Anti-inflammatory, nematicide, Insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, antihistaminic, antiacne, antiarthritic, antieczemic [18]
Oleic Acid	$C_{18}H_{34}O_2$	282.5	112-80-1	Cancer preventive, anemiagenic, insectifuge, antiandrogenic and dermatitigenic activities [25]
cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	13126-39-1	Anti-angiogenic effects and anticancer, insecticide, acaricide, herbicide, plant growth regulator antifoaming [23]
Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.4986	544-35-4	Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic [26]
Z,Z-6,13-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308.499	1000131-07-0	Antioxidant, Hepatoprotective [27]
9,12-Octadecadienoic acid ethyl ester	$C_{20}H_{36}O_2$	308.5	7619-08-1	Antiviral and anti-inflammatory activitiesto cure skin lesions Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic, flavor, hemolytic, 5-alpha reductase inhibitor [18]
2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5	1000130-90-5	Antioxidant, Antimicrobial [28]
Ethyl Oleate	$C_{20}H_{38}O_2$	310.5145	111-62-6	Anti-microbial [29]
1-Nonadecene	$C_{19}H_{38}$	266.5050	18435-45-5	Antifungal, Anticancer [17]
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.3435	4376-20-9	Antimicrobial, Antifouling [30]
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390.5561	27554-26-3	Antimicrobial, Antifouling [31]
4-Aminobenzoic acid	$C_7H_7NO_2$	137.1360	150-13-0	Antibacterial activity, antifungal activity, cytotoxicity, Schiff bases, synthesis, vitamin [32]
Lycorine	$C_{16}H_{17}NO_4$	287.3105	476-28-8	Anti-leukemia, anti-tumor, anti-angiogenesis, anti-virus, anti-bacteria, anti-inflammation, and antimalaria, but also exerts many other biological functions, such as inhibition of acetylcholinesterase and topoisomerase, suppression of ascorbic acid biosynthesis, and control of circadian period length [33]

Table 6 GCMS analysis of PZBM extract

Compound name	Molecular formula	Molecular weight	CASNo	Bio active uses
Hexenal, 2-ethyl-	$C_8H_{14}O$	126.20	645-62-5	Antifungal activity effective as an antimicrobial and acaricidal agent [34]
Uracil, 1-methyl-	$C_5H_6N_2O_2$	126.1133	615-77-0	Anti-inflamotory, Antineoplastic activity, cardiotropic, antiviral action [35]
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	28564-83-2	Antioxidant activity [17]
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126.11142000	67-47-0	Anti-carcinogenic [18]

Oxirane, [(tetradecyloxy)methyl]-	$C_{17}H_{34}O_2$	270.5	038954-75-5	Anti-angiogenic effects and Anticancer [23]
l-Sorbose	$C_6H_{12}O_6$	180.1559	87-79-6	Sugar moiety and Presevative([36]
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	57-10-3	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic,Flavor, Hemolytic inhibitor [18]
Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709	544-63-8	Antioxidant, cancer preventive, nematicide, hypocholesterolemic and lubricant [37]
9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.4455	60-33-3	Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic [18]
6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282.4614	593-39-5	Cancer preventive and insectifuge [25]
Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	57-11-4	Antiviral and anti-inflammatory activities to cure skin lesions Antioxidant, hypocholesterolemicnematicide, pesticide, anti-androgenic, flavor, hemolytic,5-alpha reductase inhibitor [18]
Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709	544-63-8	Cosmetic and topical medicinal preparation Antibacterial activity [18], [38]
Galantamin	$C_{17}H_{21}NO_3$	287.3535	357-70-0	Galanthamine, a natural product for the treatment of Alzheimer's disease Luis Marco [39]
Stigmasterol	$C_{29}H_{48}O$	412.6908	83-48-7	ovarian, prostate, breast, and colon cancers, antioxidant, hypoglycemic and thyroid inhibiting properties inhibit the absorption of cholesterol and lower serumcholesterol, anti-HIV reverse transcriptase [18]
1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.3435	4376-20-9	Antimicrobial, Antifouling activities [31]
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390.5561	27554-26-3	Antimicrobial, Antifouling activities [31]
Phthalic acid, 2-ethylhexyl hexyl ester	$C_{16}H_{22}O_4$	278.3435	1000309-02-5	Antioxidant and anticancer activities [23]
Gamma. -Sitosterol	$C_{29}H_{50}O$	414.7078	83-47-6	Hypolipidemic property [24], Antidiabetic Antimicrobial [23]
Beta.-Sitosterol	$C_{29}H_{50}O$	414.7067	83-46-5	Antilipidic, anticancer prostate [40]
Lycorine	$C_{16}H_{17}NO_4$	287.3105	476-28-8	Anti-leukemia, anti-tumor, anti-angiogenesis, anti-virus, anti-bacteria, anti-inflammation, and antimalaria, inhibition of acetylcholinesterase and topoisomerase, suppression of ascorbic acid biosynthesis, and control of circadian period length [33]

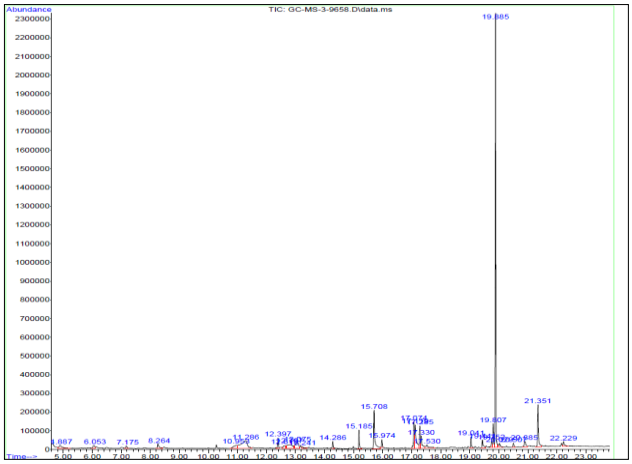

Fig 4 GCMS analysis of PZBE extract

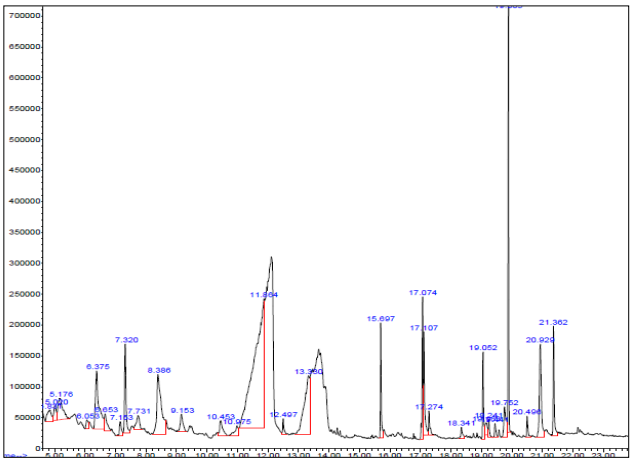

Fig 5 GCMS analysis of PZBM extract

Nitric oxide radical scavenging activity

Nitric oxide scavenging assay is presented in (Table 8). In this study, PZBE and PZBM at the concentration of 15.65, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml were studied in the nitric oxide scavenging activity. All the concentrations of PZBE and PZBM extracts have excellent nitric oxide inhibition percentage. Among this concentration, the highest inhibition percentage was observed at 1000 µg/ml concentration, with65.40±2.05 and 76.68±2.01 µg/ml ethanol and methanol extracts respectively. The second-best inhibition was recorded 56.47±0.97 and 65.15±1.58µg/ml at 500 µg/ml concentration. The IC_{50}value was represented as 349.37, 194.84, 123.78 and

116.37µg/ml PZBE, PZBM, standard ascorbic acid and rutin, respectively (Table 12). In PZBE and PZBM, nitrogen reduction capacity was noticed as extracts concentration dependent manner.

Table 7 DPPH radical scavenging activity of PZBE and PZBM

Sample concentration (µg/ml)	Antioxidant methods (Percentage of inhibition value* ± SD µg/ml)		Ascorbic acid	Rutin
	PZBE	PZBM		
1000	79.22±1.01	81.00±1.53	87.63±1.50	83.64±1.61
500	66.87±1.59	70.53±0.98	75.73±2.44	73.27±2.10
250	57.25±1.01	59.57±0.98	64.03±1.66	64.71±2.69
125	44.08±1.59	47.70±3.94	53.68±1.54	53.76±2.60
62.5	33.28±1.51	37.08±1.44	42.73±1.71	41.59±2.01
31.25	22.83±1.55	26.41±2.06	33.03±2.13	31.33±1.01
15.65	12.40±1.10	15.72±0.94	22.09±2.12	20.17±1.05

Table 8 Nitric oxide radical scavenging activity of PZBE and PZBM

Sample concentration (µg/ml)	Antioxidant methods (Percentage of inhibition value* ± SD µg/ml)		Ascorbic acid	Rutin
	PZBE	PZBM		
1000	65.40±2.05	76.68±2.01	83.46±1.62	81.25±2.41
500	56.47±0.97	65.15±1.58	71.79±1.89	70.99±1.39
250	46.42±0.99	55.18±1.52	61.39±1.95	62.80±1.63
125	36.30±1.12	45.29±1.70	49.90±1.56	51.21±1.35
62.5	26.75±1.46	35.36±2.67	42.27±1.02	42.43±1.08
31.25	18.24±1.60	24.74±1.67	32.91±1.62	33.84±1.60
15.65	10.16±1.46	14.23±2.66	20.92±2.49	22.65±1.60

Hydrogen peroxide radical scavenging activity

PZBE and PZBM extracts exhibited concentration dependent inhibition on hydrogen peroxide (Table 9), with IC_{50} values of 147.64 and 228.74 µg/ml in ethanol and methanol, respectively (Table 12). These inhibitions were significantly better than those obtained with the standard ascorbic acid and rutin (IC_{50} = 118.35 and 107.24 µg/ml). The second good inhibition was observed at 500 µg/ml concentration. When the concentration increases the percentage of inhibition also increased.

Table 9 Hydrogen peroxide radical scavenging activity of PZBE and PZBM

Sample concentration (µg/ml)	Antioxidant methods (Percentage of inhibition value* ± SD µg/ml)		Ascorbic acid	Rutin
	PZBE	PZBM		
1000	81.86±1.40	72.22±2.10	84.02±1.65	85.15±1.61
500	70.01±1.64	61.91±1.40	72.44±1.06	74.50±1.00
250	60.19±2.34	52.84±1.63	61.78±1.4	63.23±1.07
125	49.15±1.53	41.02±2.52	51.80±1.55	52.98±1.56
62.5	39.03±1.32	32.24±2.0[d]	41.65±1.67	43.12±2.08
31.25	28.33±2.05	22.15±2.09	31.53±2.64	33.06±1.61
15.65	18.01±1.45	11.12±2.15	20.11±2.47	23.75±1.53

Table 10 $ABTS^+$ radical scavenging activity of PZBE and PZBM

Sample concentration (µg/ml)	Antioxidant methods (Percentage of inhibition value* ± SD µg/ml)		Ascorbic acid	Rutin
	PZBE	PZBM		
1000	84.86±2.18	76.30±1.65	77.80±2.09	82.51±1.00
500	74.66±2.04	64.67±2.20	82.51±2.01	79.03±2.10
250	65.36±2.02	55.85±1.61	73.31±1.60	72.43±1.01
125	53.79±1.69	44.30±30	65.44±2.09	63.55±1.05
62.5	42.87±1.57	34.25±1.17	55.89±2.08	55.21±1.60
31.25	31.80±1.58	23.53±1.12	48.40±0.94	46.94±2.04
15.65	20.06±1.60	14.49±1.05	39.78±2.49	38.74±1.05

$ABTS^+$ radical scavenging activity

$ABTS^+$ radical scavenging activity of PZBE and PZBM extracts is reported in (Table 10). It was observed that when the concentration increases the absorbance value also increased. The absorbance was represents as84.86 and 76.30 at the concentration of 1000 µg/ml in PZBE and PZBM extracts, whereas the standard ascorbic acid and rutin absorbance was77.80 and 82.51 µg/ml respectively. The inhibition of both samples is more or less equal. The second excellent inhibition was recorded in 500 µg/ml concentration with 74.66 and 64.67 µg/ml, respectively against the control value ascorbic acid and rutin is 82.51 and 79.03 µg/ml. In this study results IC_{50}value of ethanol extract is 98.65 µg/ml, IC_{50}value of methanol extract is 206.43 µg/ml and IC_{50}value of ascorbic acid and rutin (standard) is47.22 and 58.17 µg/ml (Table12).

FRAP radical scavenging activity

The principle of FRAP assay is that Fe^{2+} is reduced to Fe^{3+}, and the total antioxidant capacity of the sample is represented by $FeSO_4$ as the equivalent. As presented in (Table 11), the FRAP activity of the PZBE and PZBM extracts of *P. zeylanicum* bulb recorded 79.06±2.34 and 83.39±1.00 µg/ml at 1000 µg/ml concentration were compared to standard ascorbic acid and rutin. This result is followed by the 66.73±2.02, 73.91±1.50, 76.60±1.12 and 76.53±2.03 µg/ml at 500 µg/ml concentration, in ethanol, methanol, standard ascorbic acid and

rutin respectively. The results of FRAP activity showed that the ethanol and methanol extracts of *P. zeylanicum* bulb had excellent antioxidant activity.

The most prestigious groups of secondary plant metabolites are flavonoids and phenolic acids, which exhibit a series of biological activities and certainly, have antioxidant and anti-inflammatory properties. Alkaloids, flavonoids, tannins, steroids and triterpenoids were present in both the extracts. These findings are reliable with bioactivity of *Salacia* species [41]. However, glycosides and fixed oils are present in PZBM extract, and saponins and gum and mucilage were present in PZBE extract. The anthraquinone is absent in both the extracts. The results are similar with the findings of panawala *et al*. [42]. The alkaloids, saponins and tannins are used in treating common pathogenic strains [43].

The total phenolic, flavonoid and alkaloids content of ethanol and methanol extracts have higher levels of these components. It is reported that alkaloids, phenolics, and fatty acids are some of the secondary metabolites of this plant that are responsible for its numerous medicinal properties [44]. These compounds play an important role in the antioxidant activities of several plant extracts, particularly flavonoids, which are found in high concentrations in phenolic compounds with these properties act as reducing agents, free radical scavengers, metal chelators, or deactivators of singlet oxygen and perform more than one of these functions simultaneously [45]. There are a wide variety of plant phenolic compounds known as flavonoids. These compounds are usually extremely effective antioxidants because they are hydrophobic benzene rings and have hydrogen bonding potential of OH groups, phenolic compounds often interact strongly with proteins. They are also able to act as antioxidants due to their ability to inhibit enzymes that generate radicals, such as cytochrome P450 isoforms, cyclooxygenase, lipoxygenase, and xanthine oxidase [46].

In FTIR analysis 12 and 13 different intense peaks at wave numbers which are corresponds to functional groups of the ethanol and methanol extracts of *P. zeylanicum* bulb respectively. Hence this result revealed that the ethanol and methanol extract of *P. zeylanicum* bulb have active functional groups like secondary amine, alkene, aldehyde, alkyl aryl ether, ester, aliphatic ether, halo compound, etc. These functional groups are associated with the bioactive phytochemicals in the bulb extract. This was related with the work done by Rajan *et al.* [47]. This may be due to N–H stretching and also reported to have functional groups similar to that study by Muruganantham *et al.* [48]. The presence of various essential function groups was confirmed in plants by FTIR [49].

Table 11 FRAP radical scavenging activity of PZBE and PZBM

Sample concentration (µg/ml)	Antioxidant methods (Percentage of inhibition value* ± SD µg/ml)		Ascorbic acid	Rutin
	PZBE	PZBM		
1000	79.06±2.34	83.39±1.00	85.39±1.58	87.23±2.60
500	66.73±2.02	73.91±1.50	76.60±1.12	76.53±2.03
250	56.38±1.62	63.79±1.74	65.38±1.66	66.04±1.66
125	46.63±1.00	52.63±0.99	54.70±1.12	54.30±1.64
62.5	35.24±2.05	41.69±1.03	44.88±2.70	43.78±1.64
31.25	26.04±1.39	29.66±2.57	33.34±2.18	23.66±2.11
15.65	16.15±1.61	21.48±1.98	23.62±2.774	13.95±2.57

Table 12 Inhibition concentration of fifty percentage (IC_{50}) PZBE and PZBM

Samples	Inhibition Percentage (IC_{50}) value				
	DPPH	Nitric oxide	Hydrogen peroxide	$ABTS^{+}$	FRAP
Ethanol	163.92±1.88	349.37±1.29	147.64±2.19	98.65±1.29	194.22±1.74
Methanol	147.29±2.46	194.84±0.78	228.74±0.99	206.43±1.55	117.39±2.61
Ascorbic acid	109.35±0.48	123.78±1.26	118.35±1.24	47.22±1.06	116.43±0.84
Rutin	108.22±1.47	116.37±.1.10	107.24±2.72	58.17±2.18	98.23±1.22

GCMS analysis of bulb of *P. zeylanicum* ethanol and methanol extracts showed the presence of 90 and 90 compounds at the different retention time. In both the extracts bioactive compounds are 22 and 20. The phytocompound found in both the extracts are 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl having Antimicrobial, Anti-inflammatory activity [17], 2- Furancarboxaldehyde,5-(Hydroxymethyl) consists of anti-carcinogenic [18]. Anti-inflammatory, Antibacterial, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide,Lubricant, Antiandrogenic, Flavor, Hemolytic, are found in n-Hexadecanoic acid compound [24], 9,12-Octadecadienoic acid (Z,Z) compound contain the medicinal values for Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic [18], 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester having Antimicrobial, Antifouling [30] Cytotoxic Activity.1,2-Benzenedicarboxylic acid, diisooctyl ester consists of Antimicrobial, Antifouling [31], Lycorine enclose anti-leukemia, anti-tumor, anti-angiogenesis, anti-virus, anti-bacteria, anti-inflammation, and antimalaria, but also exerts many other biological functions, such as inhibition of acetylcholinesterase and topoisomerase, suppression of ascorbic acid biosynthesis, and control of circadian period length [33].

Free radicals are generated continuously in the human body due to metabolism and diseases [50]. They can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases and extensive lysis [51]. Several synthetic drugs can be used to overcome oxidative damage, but they are associated with adverse side effects. Alternate solution to the side effects is to consume natural antioxidants from food supplements and traditional medicine [52].

DPPH is thought to be affected by antioxidants because they donate hydrogen [53]. Radical scavenging activities are very important to prevent the harmful effects of free radicals in diseases such as cancer. A well-established method for screening the antioxidant activity of plant extracts is the DPPH free radical scavenging method. The ethanol and methanol extracts of *P. zeylanicum* bulb exhibited free radical scavenging activity by inhibiting DPPH radical, which was dependent on concentrations of the extracts. The well-known antioxidant, standard ascorbic acid and rutin, showed high degree of free radical-scavenging activity than that of the plant extracts at each

concentration points. Our study is comparable to *Asteracantha longifolia* extracts showed greater scavenging effects than their standard counterparts [54]. DPPH radical scavenging property of methanolic extracts of leaves of *Pancratum quadrifolius* has been studied and it has been identified that the phenolic compounds in the extract is responsible for the activity [55]. A high phenolic content has also been found in methanolic and acetone extracts of the leaves and flowers of *P. Quadrifolius* [56]. Consequently, antioxidant activity can be attributed to phenolic compounds. Multiple reports indicate that polar solvents are effective at extracting antioxidant compounds. According to recent report, successive leaf methanolic extract fractions of *Manilkara hexandra* are the most potent antioxidant fractions [57], while ethyl acetate fraction of crude aqueous extract of *Ampelocissus latifolia* has the highest antioxidant activity [58].

$ABTS^+$cation radical scavenging activity decolorization assay associated lipophilic and hydrophilic antioxidants together, and carotenoids flavonoids hydroxycinnamates, as well as plasma antioxidants. In the present study, all the concentrations of ethanol and methanol extracts of *P. zeylanicum*have excellent nitric oxide inhibition percentage. Among this concentration, the highest inhibition percentage was observed at 1000 µg/ml concentration, in ethanol 65.40±2.05 and methanol 76.68±2.01 µg/ml respectively. The antioxidant activity of the phenolic compounds is substantially due to their redox properties [59]. Free radicals can be absorbed and neutralized singlet and triplet oxygen quenched, and peroxides decomposed by this process. Adsorption and neutralization of free radicals, quenching of singlet and triplet oxygen, or decomposition of peroxides can all be accomplished using this process. Whereas methanol and aqueous extract of Asclepiadaceae member *Hemidesmus hamiltonii* on $ABTS^+$cation radical scavenging activity IC_{50} value was 1108.9 and 2720.1 µmol/g [60]. In ethanol and methanol extracts of *P. zeylanicum* bulb, nitrogen reduction capacity was noticed as extracts concentration-dependent manner. The antioxidant activity of plant compounds against ABTS radicals appears to be better than that of DPPH radicals. This is due to more sensitivity of ABTS assay in identifying antioxidant activity which makes the kinetic reaction faster and hence results into the higher antioxidant activity [61].

Ethanol and methanol extracts of *P. zeylanicum* bulb exhibited concentration-dependent inhibition on hydrogen peroxide, with IC_{50} values of 147.64 and 228.74 µg/ml respectively. When the concentration increases the percentage of inhibition also increased. Hydrogen peroxide is an ROS (Reactive oxygen species) of great health implication because of its ability to attack, react with and penetrate biological membranes [62]. If converted to hydroxyl radical by Cu^{2+} and Fe^{2+} ions, hydrogen peroxide may also be toxic in the cell [63]. ROS such as hydrogen peroxide have the ability to penetrate biological membranes. It might be toxic to the cell, since it might be converted into other ROS, such as the hydroxyl radical [64]. It is similar to *Helichrysum stoechas* extracts are rich in phenolics that can donate an electron to H_2O_2, reducing it to H_2O. According to Saffoona *et al*. [65], caffeic acid, gallic acid, and quercetin protect mammalian cells from hydrogen peroxide-induced cytotoxicity. Taskam *et al*. [66] found that methanol extracts of *Helichrysum plicatum* exhibited moderate hydrogen peroxide scavenging activity.

It was observed that when the concentration increases the absorbance value also increased. The absorbance was represents as 84.86 and 76.30 at the concentration of 1000 µg/ml in ethanol and methanol extracts, whereas the standard ascorbic acid and rutin absorbance was 77.80 and 82.51 µg/ml respectively. It is postulated that reactive nitrogen species such as NO or peroxynitrite (ONOO-) contribute to the development of a number of diseases [67]. The inhibition of both samples is more or less equal. In this study results IC_{50}value of ethanol extract is 98.65 µg/ml, IC_{50}value of methanol extract is 206.43 µg/ml and IC_{50}value of ascorbic acid and rutin (standard) 47.22 and 58.17 µg/ml. These results almost similar to the result of radical scavenging activity in *C. flava* extract [68].

In the present study FRAP activity of the ethanol and methanol extracts of *P. zeylanicum* bulb recorded 79.06±2.34 and 83.39±1.00µg/ml at 1000 µg/ml concentration were compared to standard ascorbic acid and rutin. Wojdylo *et al*. [69] reported similar results that *Syzygium aromaticum*, out of 32 selected herbs, exhibited the highest antioxidant activity by the FRAP assay, followed by *Rosmarinus officinalis* and *Tanacetum vulgare*. The values observed with FRAP were generally low compared with related reported in other studies, Keles *et al*.[70] found that methanol extracts of *Pleurotus dryinus* have high antioxidant activity. In this results of FRAP activity showed that the ethanol and methanol extracts of *P. zeylanicum* bulb had excellent antioxidant activity. The results obtained are highly reproducible and related linearly with the molar concentration of the antioxidants present.

CONCLUSION

In conclusion, the results revealed that *P. zeylanicum* bulb ethanol and methanol extracts can provide a good source of phytochemical compounds and antioxidant showed significant effects. The activity was compared with synthetic drug shows greater percentage of inhibition of free radical. It can be used in the formulation of new drugs as well as for medicinal purposes.

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