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Comparative study of Phytochemicals, antioxidant and antibacterial activity of Phlogacanthus thyrsiformis Nees. and Justicia adhatoda Linn

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Abstract

Phlogacanthus thyrsiformis Nees. and *Justicia adhatoda* Linn., belongs to the family Acanthaceae and is a traditional medicinal plant native to Asia and widely used in medicine of Siddha, Ayurveda, Unani, homeopathy and naturopathy. The present study addresses the comparative study of phytochemicals, antioxidant, antibacterial activity and GC-MS analysis of matured dried leaves of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn., using crude extracts of acetone and methanol. The phytochemical analysis shows the presence of glycosides, tannins, proteins, triterpenoids, saponins, phenols and flavonoids in both plant extracts. Ferric chloride reducing power and DPPH radical scavenging assay were also performed to test the antioxidant activity and the IC_{50} value was also calculated and a positive result was obtained. The crude extracts were also tested against three gram negative and two gram-positive bacteria. All extracts showed dose-dependent antibacterial activity. GC-MS chromatogram analysis of the crude extracts revealed multiple peaks indicating the presence of numerous phytochemical compounds. The result clearly shows that more compounds were identified in *Justicia adhatoda* Linn. methanolic extract. In this comparative study, methanol extract was found to have more activity in testing phytochemical, antioxidant and antibacterial activity and *J.adhatoda* Linn. has greater potential medicinal value compared to *P.thyrsiformis* Nees.

Introduction

Phlogacanthus thyrsiformis Nees. and *Justicia adhatoda* Linn. gained popularity in Manipur as it is in high demand during this COVID-19 pandemic. Locals used this native herb as a folk medicine to relieve Covid-like symptoms as there is no treatment for the viral disease yet. These plants of the Acanthaceae family are evergreen shrubs native from the Indian subcontinent to Indochina, spreading across the subtropical Himalayas and Indo-Malay region, Bangladesh, Myanmar, Nepal, Thailand, Indonesia, Sri Lanka, etc. *Phlogacanthus thyrsiformis* Nees., commonly known as Nongmangkha in Manipuri, reaches a height of up to 2.4 m with leaves 13–35 cm long. The flowers are orange or brick-red in panicles up to 30 cm long. *Justicia adhatoda* Linn., commonly known as Nongmangkha-angouba in Manipuri, grows up to 3 cm in height and has leathery leaves 10–15 cm long and about 4 cm wide. Petals are white with purple streaks on the lower lip. *Phlogacanthus thyrsiformis* Nees. used to treat celiac disease, chronic bronchitis, inflammation, diabetes, jaundice, diarrhea, infectious diseases and prevent oxidative stress, asthma, rheumatism, dysentery, scabies, malaria and whooping cough, etc. It has astringent, aphrodisiac, diuretic and antipyretic properties. Likewise, *Justicia adhatoda* Linn. was used in the Ayurvedic medical system to treat various ailments such as asthma, joint pain, lumbar pain, colds, coughs, eczema, malaria, and whooping cough etc. Many medical systems, including Ayurveda, Unani, Homeopathy, Naturopathy, Siddha, and other complementary medicine systems, use plants as powerful medicines to treat a variety of dangerous diseases. In India, an estimated 80% of the population rely on herbal therapy (Hiren et al.2013). The healing effect of medicinal plants is based on the secondary plant substances they contain. The bioactive compounds produced by plants act as a defense mechanism against external stress and pathogen concerns (Tepe et al. 2005). Phytochemicals could also exhibit various bioactivities

such as: antimutagenic, anticarcinogenic, antioxidant, antibacterial and anti-inflammatory properties (Okarter and Liu, 2010). The main bioactive components in plants are alkaloids, tannins, flavonoids, phenolic compounds, terpenoids, etc. Antioxidants are a diverse group of chemicals that protect the body from oxidative damage caused by free radicals and reactive oxygen by suppressing their formation and acting as free radical scavengers. They also prevent many chronic diseases and prolong side effects (Sawa et al. 1999). Because of their reduced side effects, natural antioxidants are gaining increasing attention and are now being intensively studied (Tanizawa et al. 1992; Duh, 1998; Ali et al. 2001; Candan et al. 2003). It is known that many infectious diseases are treated with herbal extracts, which are a source of antimicrobial agents (Brantner and Grein, 1994; Somchit et al. 2003; Lee et al. 2007; Newman and Cragg, 2007). It has been reported that the leaves of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. contain important phytochemicals such as diterpene lactones, phlogantholides, flavonoids and saponins. The plant's main alkaloids, vasicin and vasicinone, are responsible for most of its activities, including antioxidant, anti-inflammatory, and bronchodilator properties, and is the area debated for many chemical compounds and pharmacological studies.

Materials and methods

Collection and extraction of crude

The fresh and matured leaves of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn., were collected from Imphal West district of Manipur, India. And the experimental works are carried out in the research laboratory of Department of Horticulture Aromatic and Medicinal Plants (HAMP), School of Earth Science and Natural Resources Management, Mizoram University located at Tanhril, Aizawl, Mizoram, during the year 2020–2022. The collected leaves of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn., were cleaned and shade dried for about 10 days and powdered using electronic grinder. Acetone and methanol were used to extract the crude by undergoing the cold maceration process. The liquid menstruum was filtered using whatman filter paper after 48 hours and allowed it to evaporate in a rotary evaporator so as to get the leaf extract in the form of residues. These residues in the form of crude were collected, dried in hot air oven and stored properly in refrigerator for further use. The aim of this study was to carried out the comparative study of phytochemical, antioxidant and antibacterial activity of the *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn.

Phytochemical screening

Preliminary phytochemical screening of acetone and methanol crude extracts of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn., were done to identify its chemical compounds present in the leaves. Screening for identification of various classes of active phytochemical constituents such as tannin, saponins, glycosides, triterpenoides and proteins were carried out using the standard protocols.

Test for tannin (Ferric Chloride Test)

For this, 2 ml of crude extract was taken and mixed with few drops of 5% ferric chloride solution. Formation of blue colour indicates the presence of hydrolysable tannins in the acetone and methanolic extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn.

Test for saponins (Frothing Test)

For this test, 0.2 ml of crude extract were taken and mixed with 0.5 ml of distilled water in a test tube and shake vigorously. Formation of stable foam indicates the presence of saponins.

Test for glycosides (Keller- Kllani Test)

2 ml of crude extract was taken and mixed with 0.2 ml of glacial acetic acid containing 2 drops of 2% ferric chloride solution. Then poured the mixture into another test tube containing 2 ml of sulphuric acid. Brown ring appearing at the interphase indicates the presence of cardiac glycosides.

Test for triterpenoides (Salkowski's Test)

2 ml of crude extract was taken in a test tube with 1 ml of chloroform and shaken. Then, few drops of concentrated sulphuric acid were added gently along with the side of the test tube. Formation of reddish-brown colour at the interphase indicates the presence of triterpenoides.

Test for proteins (Xanthoproteic Test)

For this test, 2 ml of crude extract was taken with 2 ml of sulphuric acid in a test tube and boiled in the water bath for about 10–15 minutes. Appearance of orange colour indicates the presence of protein.

Quantitative screening of phytochemicals

The quantitative screening of crude extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. were done by following the standard protocols. Readings were taken in spectrophotometer with respective nanometer and two replicates were made for each reading.

Determination of total phenol content

Total phenol content was analyzed using Folin – Ciocalteu reagent method. 1 mg of samples were taken and mixed with 1 ml of distilled H_2O. From this, 0.5 ml was taken out in a tube and 4.5ml of distilled H_2O was added again. Then, 0.5 ml of Folin – Ciocalteu reagent was added, shaken vigorously and let it rest for 5 minutes. Similarly, 0.5 ml of Folin – Ciocalteu reagent was added in the Standard Gallic acid in five different concentrations (50 µg/ml, 100 µg/ml, 200 µg/ml, 500 µg/ml and 800 µg/ml). After which 5 ml of 7% Sodium carbonate was added and let it rest for about 2 hours in dark with intermittent shaking. Absorbance was measured for both the plant samples at 517nm in spectrophotometer.

Determination of total flavonoid content

Test for flavonoids was performed using Aluminium chloride. 2 mg of samples were taken and 2 ml of distilled H_2O was added. From this, 0.5 ml was taken out in a test tube and 0.5 ml of Alcl3 was added to make up to 1ml volume. Then the tubes are kept in dark for about 10 minutes. Development of orange-

yellowish colour indicates the presence of flavonoids. Quercetin was used as standard in different concentrations (50 µg/ml,100 µg/ml, 200 µg/ml, 500 µg/ml, 800 µg/ml) as standard with methanol making up to 1ml volume. Absorbance was measured in spectrophotometer in 415nm.

GC-MS analysis of the plant extracts

GC-MS was used for identification of major bioactive compounds present in the crude methanolic extract of *Phlogacanthus thyrsiformis* and *Justicia adhatoda* Linn. GC-MS was carried out using the method GC: Baanu GC mth and MS: Baanu MS EXP. This system is composed of a gas chromatograph connected to a mass spectrometer (GC-MS) instrument in which the samples were analysed by using software Turbomass. 2µL of each plant extract were injected in the split mode by using helium as a carrier gas. Column length of 30.0m and film thickness of 250µm was used. The total run time was 30.0min with an injection split ratio of 20:1. For the oven program, the initial temperature was 40°C for 5 min, ramp 10°C/min to 150°C, hold for 5 min, ramp 40°C/min to 250°C and hold for 8 min. Injector temperature was 220°C, solvent delayed = 3.00min, transfer temperature = 180°C, Source temperature = 180°C and scan 50 to 500 Da. The identification of the various components was accomplished using computer searches in NIST library.

Antioxidant activity

The antioxidant activity of the *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. were determined using two different methods.

DPPH assay

Radical scavenging activity were determined by calorimetric assay using 11-diphenyl-2-picrylhydrazyl (DPPH) as a source of free radical according to the method of Blois (1958). Five different concentrations (20µl/ml, 40 µl/ml, 60 µl/ml, 80 µl/ml, 100µl/ml) of standard Quercetin and the plant samples were taken and diluted with methanol making up to 1ml and then, 1 ml of DPPH was added, shaken and rest it for 30 mins. at room temperature. The absorbance was recorded at 517nm in UV Spectrophotometer. The percentage inhibition and IC_{50} were calculated.

The DPPH scavenging activity (% inhibition activity) was calculated using the equation:

$$\text{DPPH \% scavenging} = \frac{\text{Control absorbance - Sample absorbance x100}}{\text{Control absorbance}}$$

Reducing power of $FeCl_3$

Antioxidant activity of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. were also determined by reducing power assay method. Five different concentrations (10µl,30µl,50 µl,70 µl, 100µl) of the standard gallic acid and the plant samples were taken in a test tube and diluted with DMSO to make upto 1ml volume. Then, 2.5 ml of phosphate buffer was added in each tube followed by adding 2.5ml of 1% potassium ferricyanide, well shaken and taken to water bath at a control temperature of 50°C

for about 20 minutes. After which 2.5 ml of 10% trichloroacetic acid (TCA) was added in the mixture. Later, 2.5 ml of supernatant layer of the mixture was taken out in different test tubes and diluted with 2.5 ml of distilled water. Lastly, 0.5 ml of 1% ferric chloride is added. The absorbance was taken at 700 nm in UV Spectrophotometer.

Antibacterial activity

Disc diffusion method which is also known as Kirby- Bauer test was performed to determine the antibacterial potential of the crude extracts. Two gram positive and three gram-negative bacteria were taken for study viz., *Bacillus cereus* (MTCC-430), *Staphylococcus aureus* (MTCC-6908), *Escherichia coli* (MTCC-723), *Salmonella typhimurium* (MTCC-3224) and *Vibrio cholerae* (MTCC-3906). Suspensions of microbial strains with an optical density of McFarland were made in distilled water. Sterile filter paper discs were cut out and impregnated with the different concentrations of crude and placed on the agar medium in the petri dishes along with positive and negative control. Rifampicin was used as a positive control and DMSO was used as a negative control. Inverted discs were then placed in the incubator at 35°C within 15 minutes and the plates are examined after 18–24 hours. The diameter of the zones was recorded to calculate the zone of inhibition of the mentioned bacteria against *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn.

Results and discussion

Qualitative Phytochemical Screening

Results of the phytochemical screening of acetone and methanol leaf extracts of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. were showed in the table below.

Table 1
Phytochemical screening of acetone and methanol leaf extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn.

Sl. No.	Phytochemical compounds	Result	
		Acetone extract	Methanol extract
1.	Tannin		+
2.	Saponin	++	++
3.	Glycosides	++	++
4.	Protein		+
5.	Triterpenoids tests	++	++

- indicates absence, + indicates minimal presence, and + + indicates maximum presence

In the present qualitative phytochemical test, saponin, glycosides, triterpenoides, tannin and protein are found in the plant extracts. Comparing the leaf extracts of acetone and methanol, the methanol leaf extracts showed profound activity in all the test results. Similar bioactive components such as terpenoids, phenols, tannins, proteins and saponins were also obtained by (Gohel et al.2021) in the methanol leaf extract of *Adhatoda vasica* Nees. Similar result was also obtained by (Mohd et al.2016 and Nongthombam et al.2018) in the leaf extract of *Phlogacanthus thyrsiformis* Nees. The result of the above study was also in conformity with the earlier study of (Malathi et al. 2018) which revealed the presence glycosides, tannins and proteins in acetone and methanol leaf extracts.

Quantitative phytochemical screening

Total phenol content

The acetone leaf extracts of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. was found to contain 37.8 ± 0.005 and 33 ± 0.001 CE mg/100g of total phenols respectively. And the methanolic leaf extract of *Phlogacanthus thyrsiformis* Nees. was found to contain 42.4 ± 0.02 CE mg/100g and *Justicia adhatoda* Linn. was found to contain 39.2 ± 0.001 CE mg/100g. This study shows that the total phenol content in the methanolic leaf extract of *Phlogacanthus thyrsiformis* Nees. is more compared to *Justicia adhatoda* Linn. Similar result was also showed by (Kripasana and Xavier. 2020) in the phytochemical analysis of methanol extract of *Adhatoda vasica* Linn. and *Phlogacanthus thyrsiformis* Nees. Highest amount of Phenol was found to be present in *Phlogacanthus thyrsiformis* Nees. (Chanu et al. 2011) also showed the presence of phenol in similar quantity (48.75 ± 1.43 mg/g) in the methanol leaf extract of *Phlogacanthus thyrsiformis* Nees. In a previous study carried out by (Naqvi et al. 2013) the total phenol content in methanol extract of *Justicia adhatoda* Linn. was found to be 30.4 ± 3.3 mg/100g in dry extract. This result is also in close conformity with the present study.

Total flavonoid content

The presence of total flavonoid in acetone leaf extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. were found to be 35.5 ± 0.008 and 10.65 ± 0.004 mg/100g respectively. And the presence of total flavonoid in methanolic leaf extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. was found to be 51.75 ± 0.044 mg/100g and 98.75 ± 0.005 mg/100g respectively. The obtained R^2 value of 0.9903 shows that the linearity of the presented graph was validated. Presence of total flavonoid is found to be higher in methanol leaf extract of *Justicia adhatoda* Linn. In a study conducted by (Das et al. 2015) to investigate the potential role of a methanol extract from *Phlogacanthus thyrsiformis* Nees. leaves, flavonoid were discovered in trace concentrations. Another investigation was done by (Gamage et al. in 2021) to determine the total flavonoids content in the ethanolic leaf extract of *Justicia adhatoda* Linn. The total flavonoid was discovered to be 115.63 ± 0.93 under shade drying conditions. This outcome is reasonably close to the outcome of the current investigation.

GC-MS analysis

GC-MS analysis was undertaken to detect the phytochemical constituents. Chromatogram analysis of the methanolic extracts of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. showed several peaks that indicated the presence of numerous phytochemical compounds. In this study, *Phlogacanthus thyrsiformis* Nees. showed the presence of 11 compounds and *Justicia adhatoda* Linn. showed the presence of 18 compounds. However, in both the samples, the highest peak of the chromatogram exhibits the presence of Neophytadiene, a compound which is reported to possess antibacterial activity, anti-inflammatory, for treatment of various ailments. Presence of Phytol and other comparable compounds were also identified by (Suchiang and Kayde, 2017) in the methanol extract of *Phlogacanthus thyrsiformis* Nees. Compounds such as Phytol and Neophytadiene were also identified in a study conducted by (Kripasana and Xavier 2020) and (Deka et al., 2023) in the extract of *Phlogacanthus thyrsiformis* Nees. In a comparative study conducted by (K Srinivasan et al., 2015) similar compounds such as Dimethyl, Dimethylamanioethyl Ester were also discovered from the extract of *Justicia adhatoda* Linn. A study to isolate possible phytocompounds from *Justicia adhatoda* Linn. was also carried out by (Khan and Bhadauria 2017). Similar compounds such as Dimethyl and Bis (Tert-Butyldimethylsilyl)2,3-Bis (Tert-Butyldimethylsilyl) were also identified. Neophytadiene, the compound that exhibits the highest peak in the current investigation, was also previously discovered in *Justicia adhatoda* Linn. leaf extracts by (Kripasana and Xavier 2020). In comparison to other plants in the Acanthaceae family, the study also demonstrates that the leaf extract of *Justicia adhatoda* Linn. contains a higher concentration of phytochemical components. This outcome closely aligns with the current research since the maximum number of chemical compounds were isolated from the leaf extract of *Justicia adhatoda* Linn. The active constituents with their retention time (RT), Compound name, molecular formula and molecular weight are given in Table 2 and 3. The GC-MS chromatogram are given in Figs. 5 and 6.

Table 2
GC-MS analysis of methanolic crude extract of *Phlogacanthus thyrsiformis* Nees.

Sl.no.	RT	COMMON NAME	FORMULA	M.W.
1	11.25[a] 12.43[b]	Spiro [2,4] Heptane ,1 ,1-Dichloro	$C_7H_{10}C_{12}$	164
2	23.44	Phytol	$C_{20}H_{40}O$	296
3	23.76	Fumaric Acid, Heptyl 3- Methylbut-3-Enyl Ester	$C_{16}H_{26}O_4$	282
4	23.83	4- Methyl – 2, 4-BIS (P- Hydroxy phenyl) Pent – 1-ENE, 2 TMS derivative	$C_{24}H_{36}O_2S_i2$	412
5	24.11	3-Pentanol, 3- Methyl-	$C_6H_{14}O$	102
6	24.15	1,3 –Dioxolane, 2- Pentadecyl-	$C_{18}H_{36}O_2$	284
7	24.30	Allo –Inositol	$C_6H_{12}O_6$	180
8	24.76	Neophytadiene	$C_{20}H_{38}$	278
9	26.94	Tetrasiloxane, Decamethyl –	$C_{10}H_{30}O_3S_i4$	310
10	26.96[a] 28.73[b]	Anthracene, 9 –Ethyl – 9, 10 –Dihydro – 10-Trimethylsilyl	$C_{19}H_{24}Si$	280
11	29.17[a] 30.88[b]	1,4 – Cyclohexadiene, 1,3,6-TRIS (Trimethylsilyl)	$C_{15}H_{32}S_i3$	296
Where, RT = Retention Time, MW = Molecular Weight				

Table 3
GC-MS analysis of methanolic crude extract of *Justicia adhatoda* Linn.

Sl.no.	RT	COMMON NAME	FORMULA	M.W.
1	9.17[a] 9.19[b]	Peroxide, Dimethyl	$C_2H_6O_2$	62
2	9.22	Dimethyl sulfoxide	$C_2H_6O_S$	78
3	9.40	Dimethylsulfoxonium Formylmethylide	$C_4H_8O_2S$	120
4	11.26	D-Limonene	$C_{10}H_{16}$	136
5	11.63	Ethyne, Difluoro-	C_2F_2	62
6	12.44	1-Bromo – 3-Butene – 2-OL	C_4H_7OBr	150
7	14.46	Bismuthine, Tripropyl	$C_9H_{21}B_i$	338
8	23.45	Neophytadiene	C_2OH_{38}	278
9	23.50	Cyclobutanecarboxylic Acid,2- Dimethylamanioethyl Ester	$C_9H_{17}0_2N$	171
10	23.65	1,3,5- Triazine – 2, 4 –Diamine, N (2), N (4)	$C_8H_{12}N_8S$	252
11	24.11	3-Methyl – 2-(2-Oxopropyl) Furan	$C_8H_{10}O_2$	138
12	24.25	2-Ethylhexanal Ethylene Glycol Acetal	$C_{10}H_{20}O_2$	172
13	24.76	Neophytadiene	$C_{20}H_{38}$	278
14	24.89 24.95	1,3,5- Trioxane, 2,4,6-Tripropyl -	$C_{12}H_{24}O_3$	216
15	25.15	1,3-Dioxolane, 2 – Pentadecyl -	$C_{18}H_{36}O_2$	284
16	25.65	Anthracene, 9 – Ethyl – 9, 10 – Tripropyl -	$C_{19}H_{24}Si$	280
17	30.10	Tetrasiloxane, Decamethyl-	$C_{10}H_{30}O_3Si_4$	310
18	31.13	Bis (Tert-Butyldimethylsilyl)2,3-Bis (Tert-Butyldimethylsilyl) Oxy	$C_{28}H_{60}O_6Si_4$	604
Where, RT = Retention Time, MW = Molecular Weight				

Figure 6. GCMS chromatogram of methanolic extract of *Justicia adhatoda* Linn.

Determination of antioxidant activity

DPPH

The percentage inhibition of the DPPH radical by methanol leaf extract of *Phlogacanthus thyrsiformis* Nees. *and Justicia adhatoda* Linn. was recorded as 44.06% and 48.31% and acetone leaf extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia ahatoda* Linn. plants were recorded as 42.94% and 44.92%. Percentage of inhibition for standard quercetin was found to be 51.69%. The IC_{50} value obtained for the standard quercetin is 92.15 µg/ml at the maximum concentration. IC_{50} value of methanol leaf extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. at the maximum concentration was found to be 5.79 µg/ml and 5.14 µg/ml respectively. IC_{50} value of acetone leaf extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. at the maximum concentration is 5.71 µg/ml and 5.66 µg/ml. The least IC_{50} was showed by the crude extract of *Justicia adhatoda* Linn., therefore it exhibits the highest antioxidant property. The antioxidant potential of methanolic leaf extracts of *Phlogacanthus thyrsiformis* Nees. was examined in a study by (Nongthombam et al. 2018), using the DPPH test. The IC_{50} value was 7.86 ± 0.35mg and the DPPH inhibition percentage in 100 mg of dry leaf was found to be 85.6 ± 1.5. Similar study was also conducted by (N. Saran et al. 2019) in the ethanolic extract of *Justicia adhatoda* Linn. where the DPPH inhibition was found to be 86.5 ± 0.25 at 250µg/ml conc. These earlier findings demonstrated higher antioxidant capacity of *Justicia adhatoda* Linn. Standard graph and graphical representation of DPPH assay is given below.

Reducing power $FeCl_3$

The absorbance was found to increase with the increase in concentration of the plant extract which indicated increased reducing power; thus, proving the antioxidant property. The methanol extract of *Justicia adhatoda* Linn. showed higher reducing power. The standard graph of acetone and methanol crude extract and Graphical representation of reducing power of *Phlogacanthus thyrisiformis* Nees. and *Justicia adhatoda* Linn., against standard gallic acid is given below. The antioxidant ability of certain compounds with their reducing power was observed by (Rajurkar et al.2012) and it was found that the reducing power of *Justicia adhatoda* leaves extract shows maximum reducing power [38.683 mg equi of Fe + 3 /g of dw]. Standard graph and graphical representation of the present study are given below.

Determination of antibacterial activity

The acetone crude extract of *Phlogacanthus thyrsiformis* Nees. shows maximum zone of inhibition against the bacteria in the order *Staphylococcus aureus > Bacillus Cereus > Vibrio cholerae > Salmonella typhimurium > Escherichia coli*. Antibacterial activity of methanolic crude extract of both the plants against three gram negative and two-gram positive bacteria showing their mean of ZOI (zone of inhibition) is given below.

Table 4
Antibacterial activity of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn.

Bacteria	*Phlogacanthus thyrsiformis* Nees.		*Justicia adhatoda* Linn.	
	RIFAMPICIN	Max. conc. 300µg/ml	RIFAMPICIN	Max. conc. 300µg/ml
Bacillus cereus (MTCC-430)	27mm	10.25 ± 0.43mm	28.5 ± 0.5mm	10.5 ± 0.5mm
Escherichia coli (MTCC-723)	28mm	-	27.5 ± 0.5mm	-
Salmonella typhimurium (MTCC-3224)	28mm	10.5 ± 0.5mm	28mm	10.5 ± 0.5mm
Vibrio cholerae (MTCC-3906)	30mm	10mm	27mm	10.75 ± 0.25mm
Staphylococcus aureus (MTCC-6908)	28mm	11 ± 0.71mm	26.5 ± 0.5mm	11.5 ± 0.5mm

Data is represented as Mean ± SD. of the zone of inhibition.

Antibacterial activity in methanol crude extract of *Justicia thyrsiformis* Nees. and *Justicia adhatoda* Linn. showed more activity compared to acetone crude extract against the tested pathogens. *S. aureus* showed the maximum zone of inhibition and *E. coli* showed the minimum zone of inhibition. (Karthikeyan et al. 2009) also reported similar results of the crude ethanolic extract of *Adhatoda vasica* Nees. leaf, where he mentioned that *Escherichia coli* show no activity against the leaf extract as compared to *Staphylococcus aureus*, showing maximum zone of inhibition. In a study conducted by (Sarbadhikary et al. 2015), similarly *Phlogacanthus thyrsiformis* Nees. showed no activity against *E. coli*. (Rajmuhon et al. 2010) also showed similar results in the leaf extracts of *Phlogacanthus thyrsiformis* Nees., a broad spectrum of activity was showed against most of the bacterial strain and *Staphylococcus aureus* exhibited a maximun diameter of zone of inhibition in the methanol extract.

Conclusion

The study revealed that the two traditional medicinal plants *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. showed pharmacological activities, potential antioxidant and antibacterial properties indicating its protective role against microbial infections and oxidative damage. The GC-MS analysis of the two plant extracts also exhibited the presence of various phytoconstituents that possess antibacterial, antioxidant and anti-inflammatory activities. From the results, it can be concluded that the methanolic dried matured leaf extract of *Justicia adhatoda* Linn., was found to be more active compared to the other extracts. However, the plant extract of *Phlogacanthus thyrsiformis* Nees., also exhibits active pharmacological properties. Both the plants confirmed their therapeutic and combating abilities which can be use against multiple diseases. Further synthesis of active phytochemicals can lead to the

development and discovery of novel herbal drugs. However, the fact that regional and seasonal fluctuation play a big role in the chemical composition, discrepancies, necessitating the authentication of chemical constituents, pharmacological potentials and production processes of the compounds, different studies show different results. A detailed in vivo study needs to be conducted for validation of activities to develop novel herbal drugs from these two plants.

Declarations

Conflict of interest

The authors declared that they have no conflict of interest.

Author Contribution

T.N. and A.Y. conducted the chemical analysis. T.L. and A.K. prepared the figures and tables. Main script was written by T.N. and H.S. All authors reviewed the paper.

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Figures

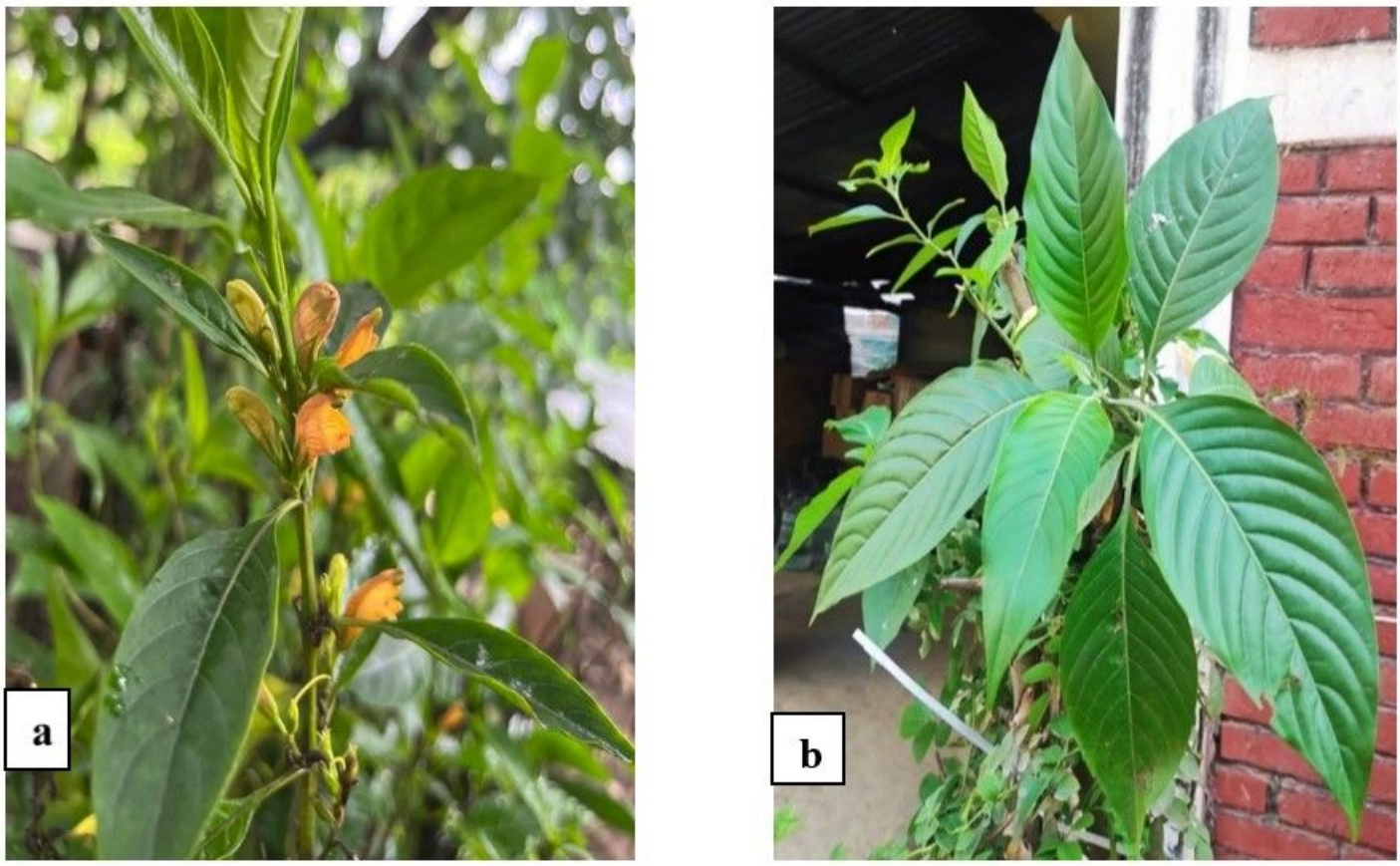

Figure 1

(a) *Phlogacanthus thyrsiformis* Nees. plant with inflorescence and (b) *Justicia adhatoda* Linn. plant

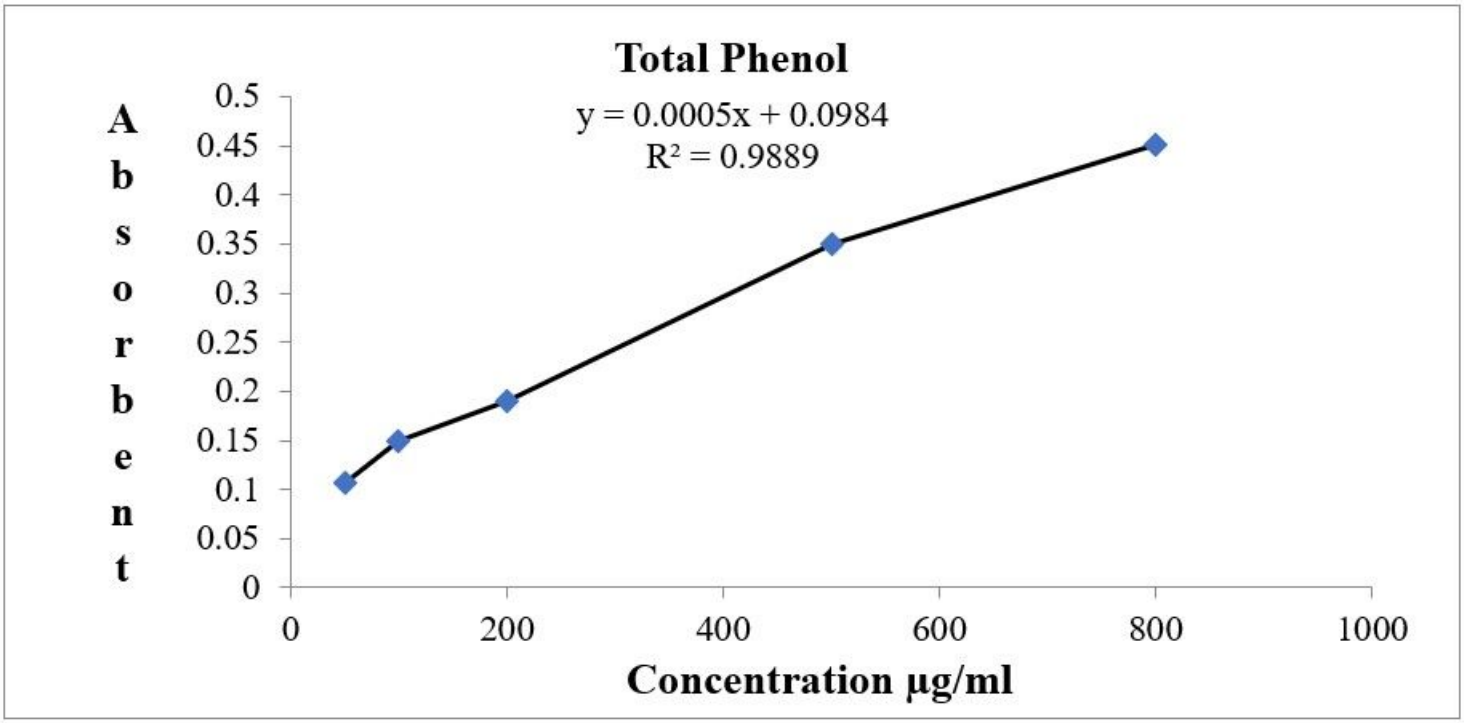

Figure 2

Graphical representation of Total Phenol content in acetone extract

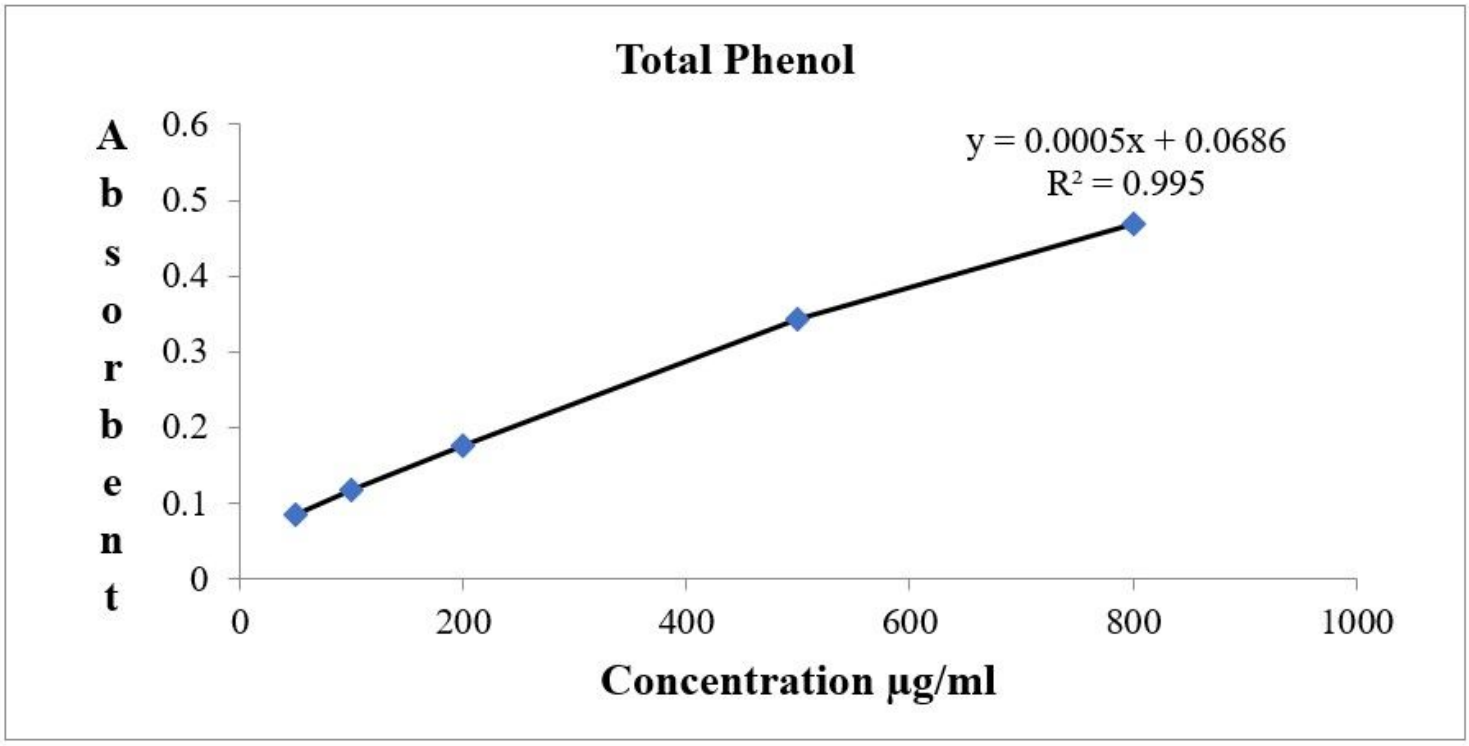

Figure 3

Graphical representation of Total Phenol content in methanol extract

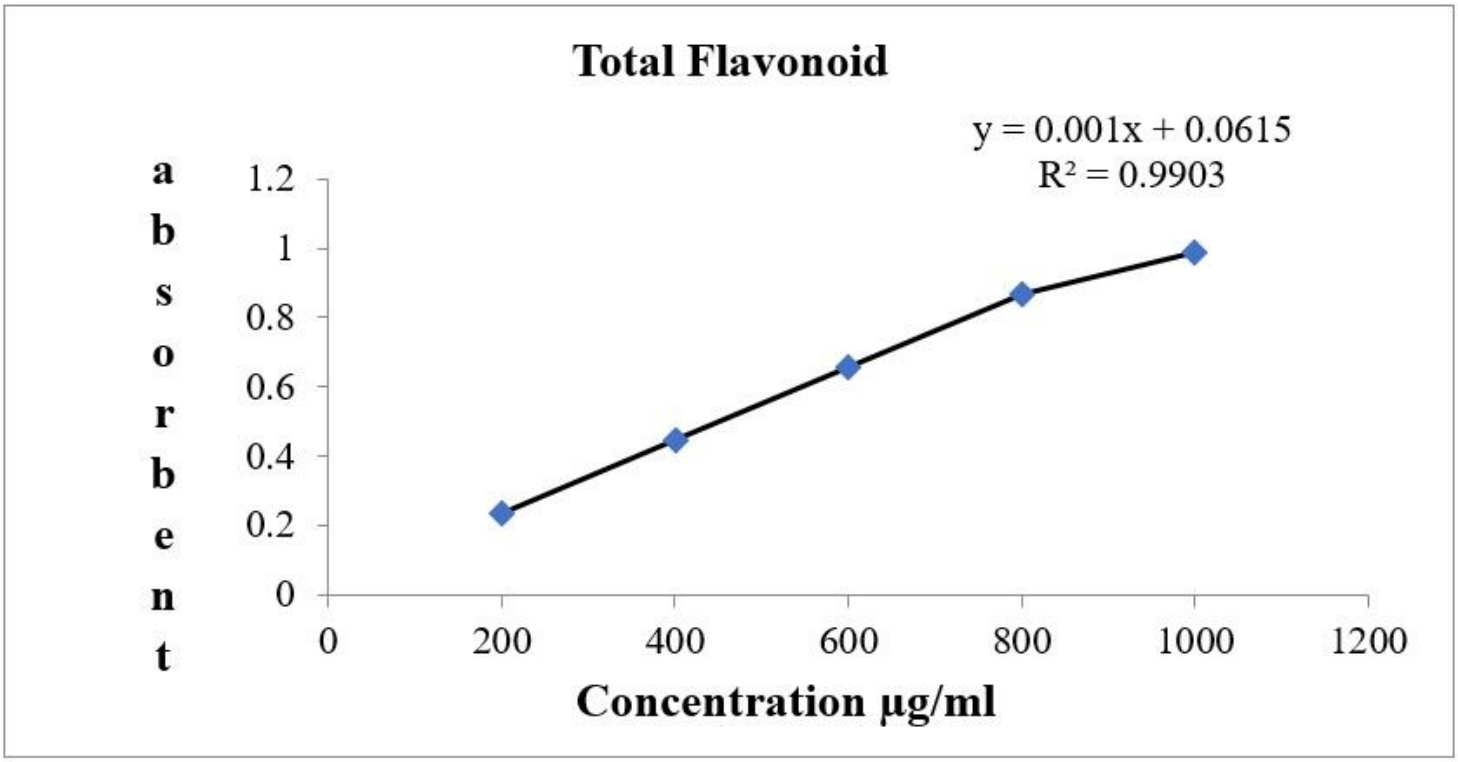

Figure 4

Graphical representation of standard Quercetin for total flavonoid

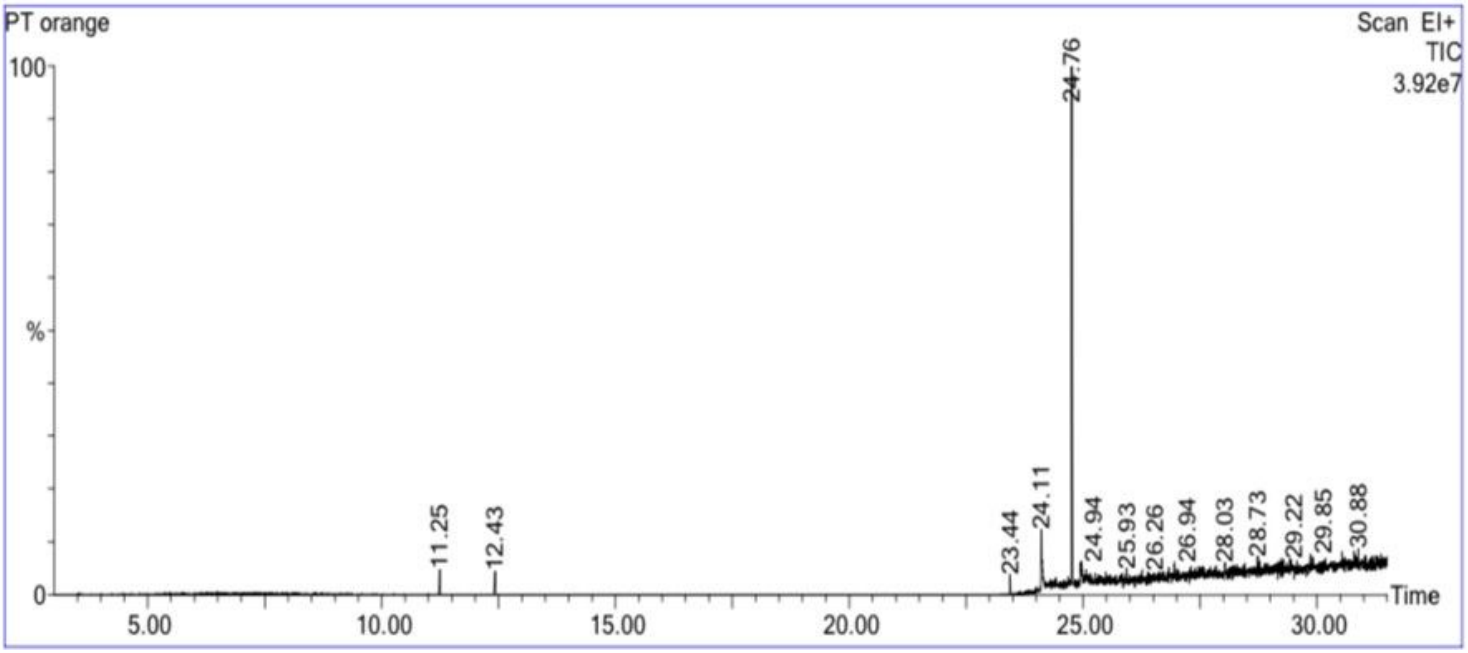

Figure 5

GCMS chromatogram of methanolic extract of *Phlogacanthus thyrsiflorus* Nees.

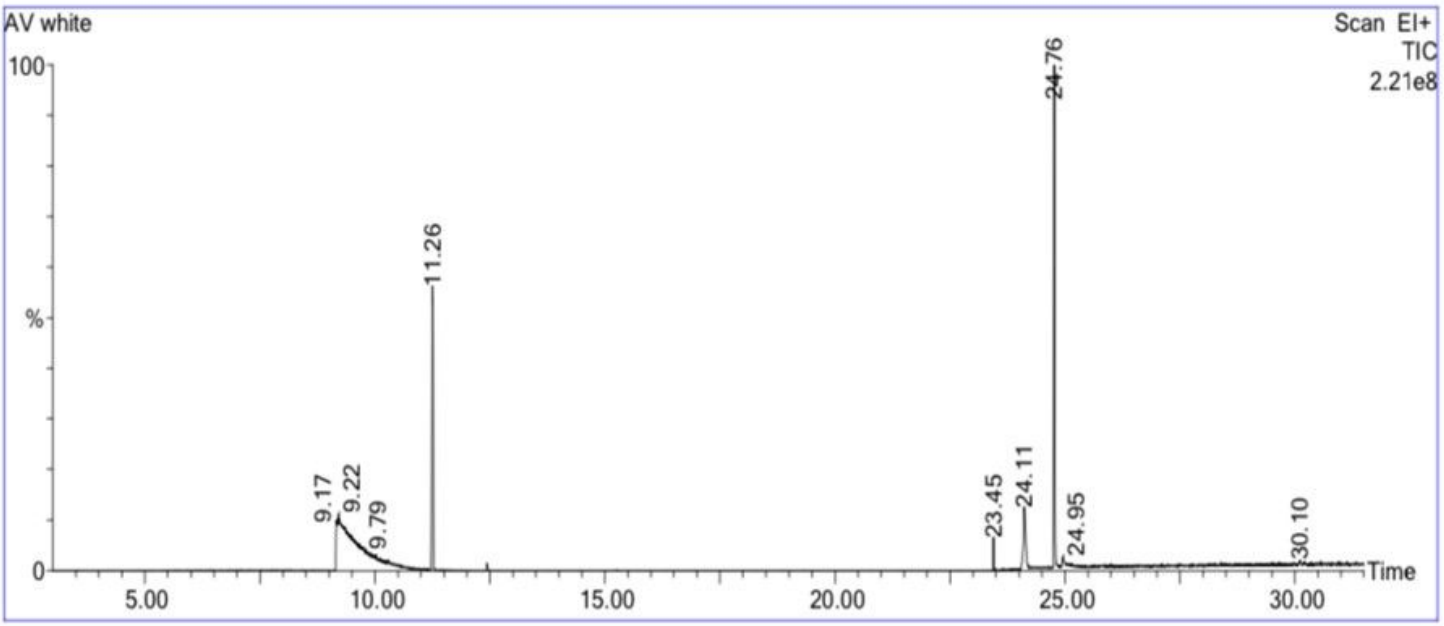

Figure 6

GCMS chromatogram of methanolic extract of *Justicia adhatoda* Linn.

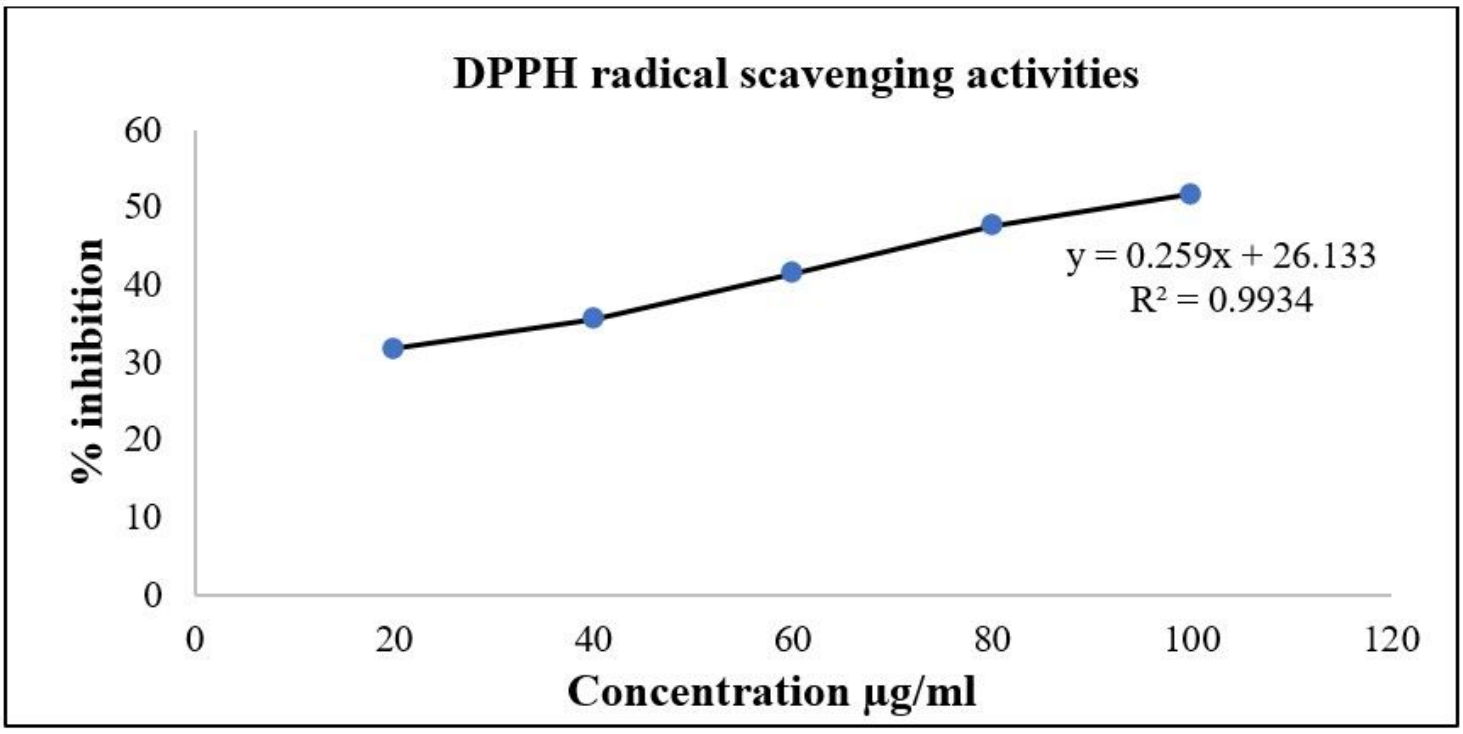

Figure 7

Figure 6. Inhibition % of standard quercetin by DPPH assay

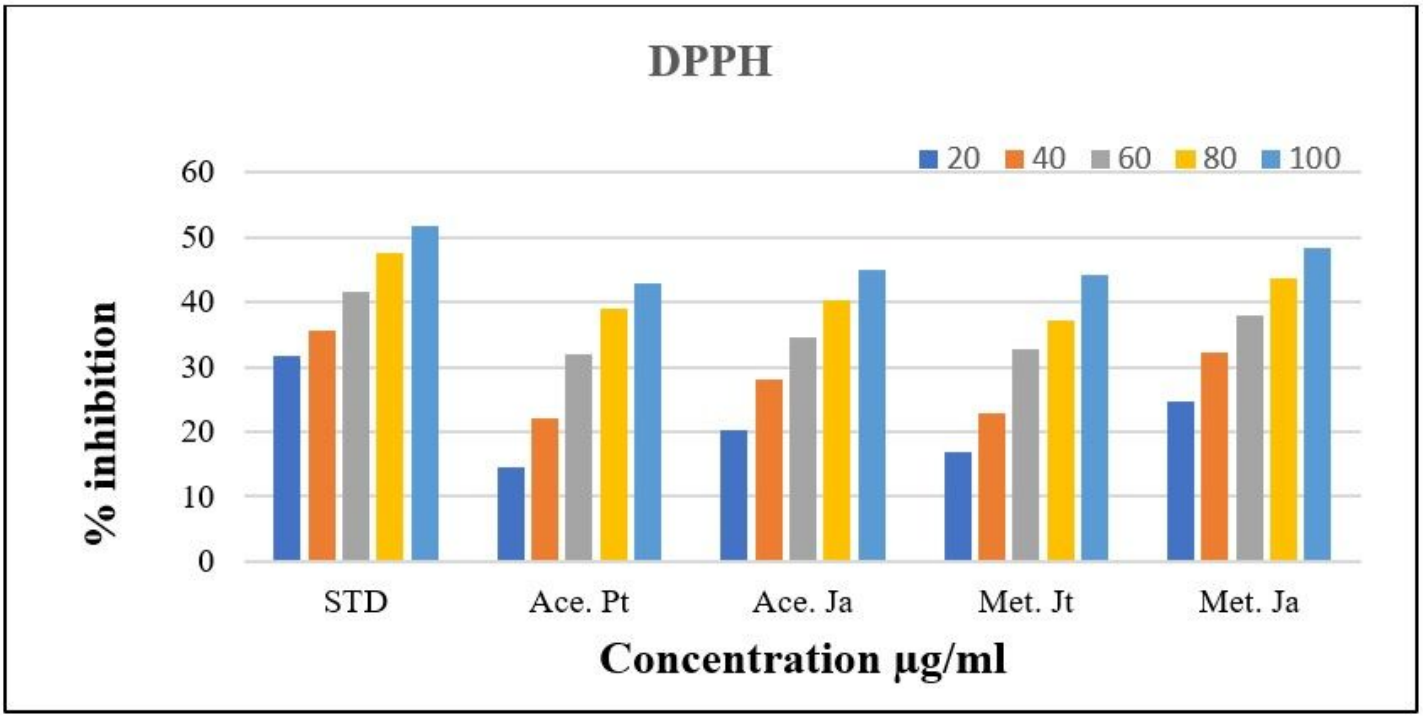

Figure 8

Figure 7. Graphical representation of DPPH radical scavenging activity

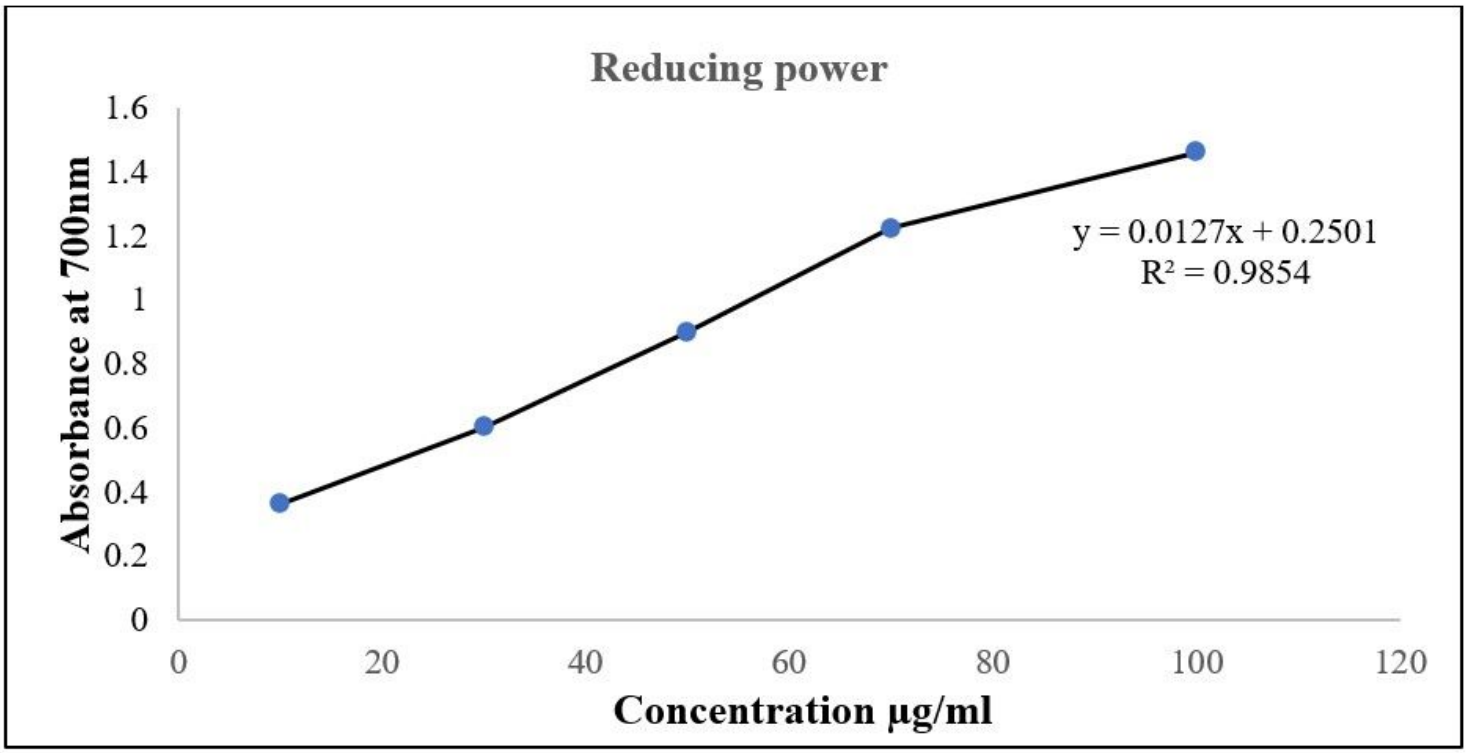

Figure 9

Figure 8. Reducing Power Standard graph of acetone crude extract

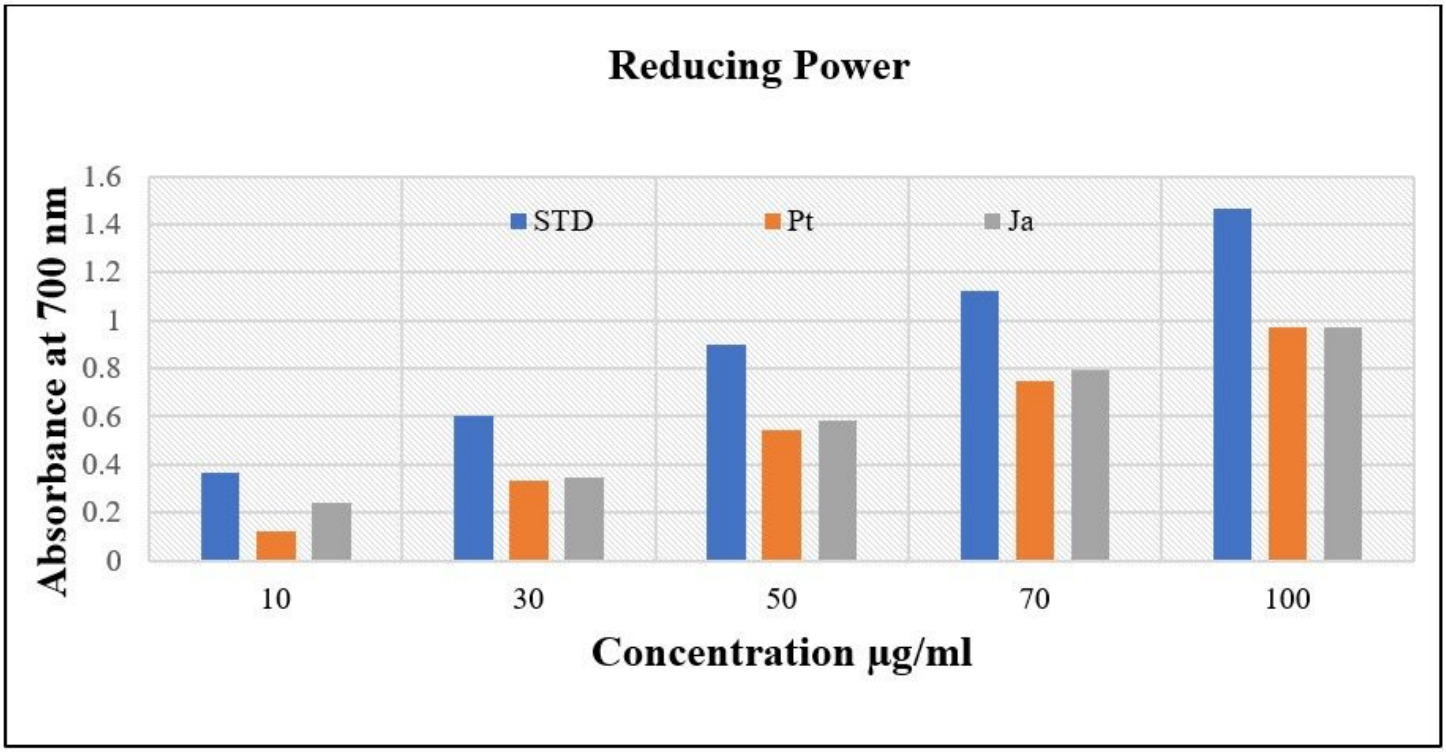

Figure 10

Figure 9. Graphical representation of reducing power of Acetone extract of *Phlogacanthus thyrisiformis* Nees. and *Justicia adhatoda* Linn. against standard gallic acid

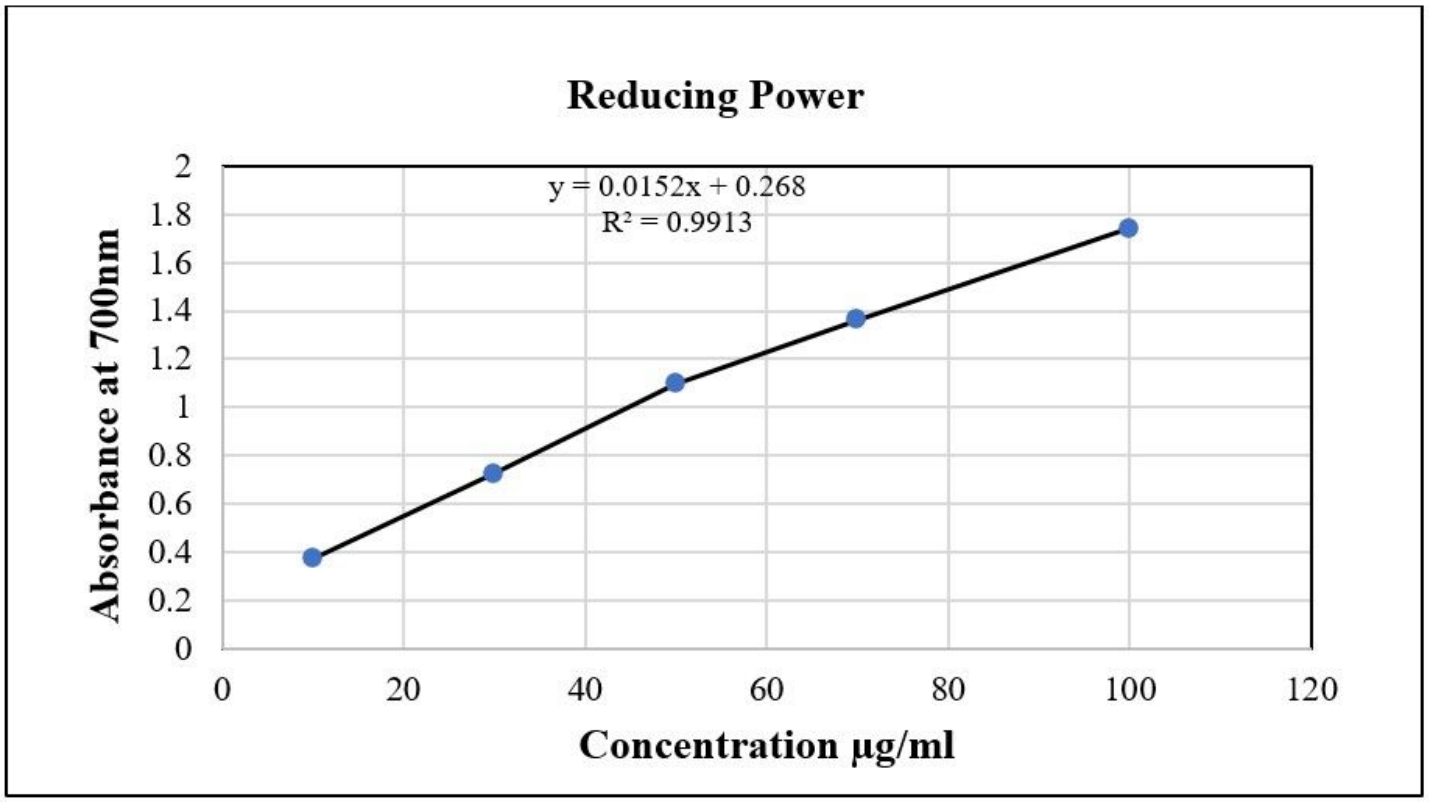

Figure 11

Figure 10. Reducing power standard graph of methanol crude extract

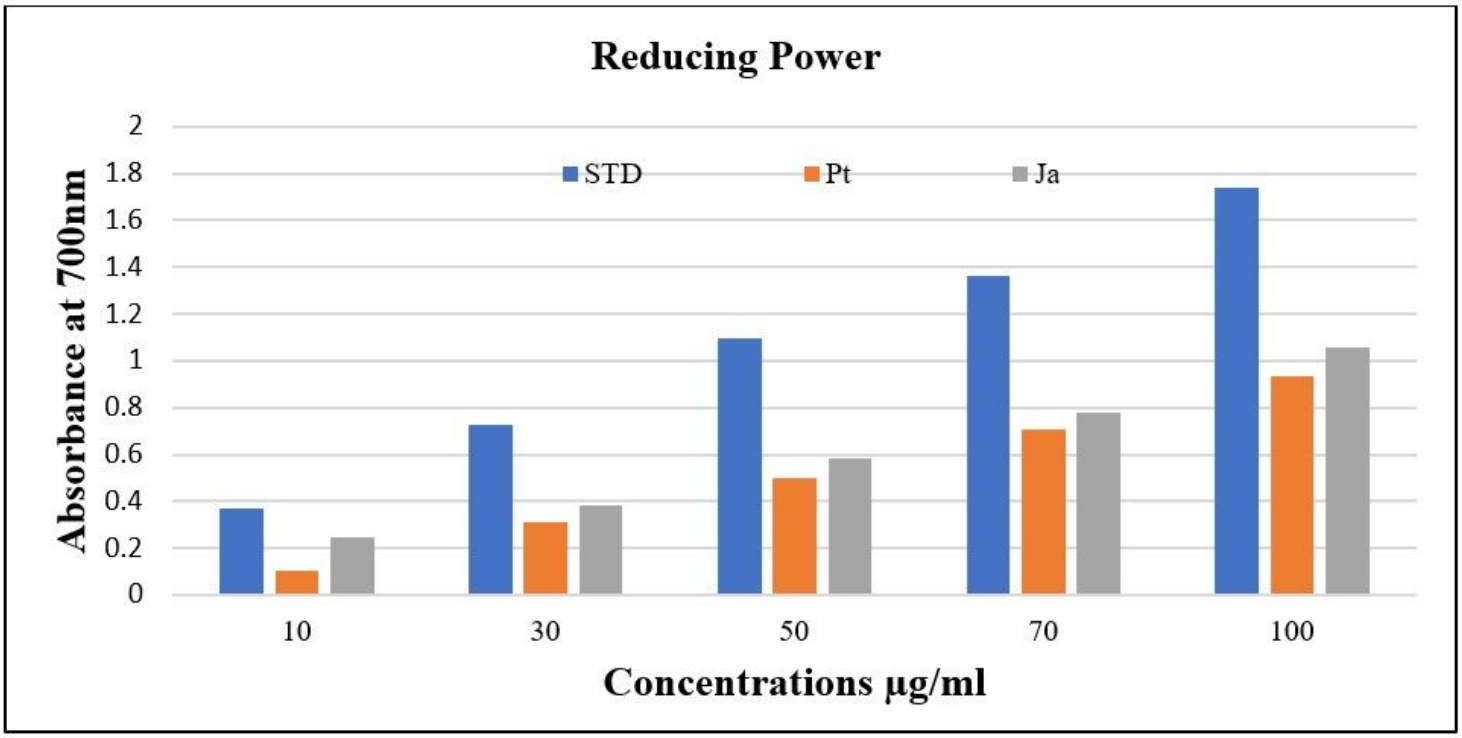

Figure 12

Figure 11. Graphical representation of reducing power of methanol crude extract of *Phlogacanthus thyrsiformis* Nees. and *Justicia adhatoda* Linn. against standard gallic acid.