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Antioxidant, Antimicrobial, and Phytochemcial Assessment of *Tephrosia purpurea* (L.) Pers. Extracts

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ABSTRACT

Tephrosia purpurea is a traditional herbal medicine used in impotency, asthma, dyspepsia, hemorrhoids, gonorrhea, rheumatism, enlargement of kidney and spleen. The present study deals with the in vitro evaluation of antioxidant, antimicrobial activities and phytochemical profiling of methanolic and ethanolic leaf extracts of *T. purpurea*. Antioxidant activity was analyzed by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and α-diphenyl-β-picrylhydrazyl (DPPH) assays while antimicrobial activity was evaluated by disc diffusion assay and Minimum Inhibitory Concentration against four bacteria and two fungi. Extracts were subjected to phytochemical profiling utilizing Fourier Transform Infrared Spectroscopy and Liquid Chromatography- Mass Spectrometry. Both the extracts showed considerable free radical scavenging activity with IC_{50} value of 78.7, 49.7 and 65.38, 55.38 µg/ml against DPPH and ABTS, respectively. The extracts exhibited both antibacterial and antifungal activities against the tested microorganisms however *Staphylococcus aureus* and *Bacillus subtilis* were the most susceptible bacteria towards the extracts. The extracts showed concentration dependent inhibition of microorganisms. The LC-MS analysis identified 37 phyto compounds in both the extracts of which 5-Methyl-2-[5-(2-methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-Phenol, 3b,4,5,6,7,7a,9,10,11,12-Decahydrobenzo[b] fluoranthene and N-(3-Amino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-N-phenylacetamide were reported for the first time from the plant. FTIR data revealed the presence of several phytocompound classes, notably flavonoids, saponins, glycosides, phenolics, steroids and tannins. This baseline study is a notable step towards the exploration of *T. purpurea* leaves as an economical natural source of broad-spectrum antimicrobial and antioxidant agents.

Key words: *Phytochemistry, Microbial resistance, Medicinal plant, Antioxidants, Tephrosia purpurea*

Introduction

Plants have been used as a source of medicine and for maintaining wellbeing by humans since prehistoric times. Various traditional preparation of plants prepared worldwide have been known to exhibit numerous biological activities especially antimicrobial, antifungal, antibacterial, and antioxidant (Pasdaran *et al*., 2017; Kýlýç *et al*., 2017). These traditional preparations have different categories of bioactive phytocompounds which render numerous pharmacological activities and have paved the way for the development of modern drugs (Atanasov *et al*., 2015). More than 50 % of the contemporary drugs are products of natural origin and play a pivotal role in pharmaceutical industries (Chopra and Dhingra, 2021).

The development of multidrug resistance by pathogenic microorganisms against synthetic drugs has become a global concern. The recent developments in the field of active phytocompound assisted treatment of infectious diseases have gained interest

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of researchers (Prestinaci *et al*. 2015). Novel compounds from natural sources are being identified and given priority over synthetic antibiotics. Primarily due to increased awareness of limited efficacy against major diseases and side effects arising from their use. Antioxidants play a critical role in rendering protection against oxidative stress and disorders arising from free radical damage. The existing synthetic antioxidants like citric acid, propyl gallate, Butylatedhydroxyanisole (BHA), Butylatedhydroxytoluene (BHT), and ascorbic acid have been reported to have side effects and toxicity (Rice-Evans and Packer, 2003). Consequently, the interest in natural sources of antioxidant has been increasing. Due to rapid emergence of multi-drug resistance among pathogens and side effects associated with synthetic antioxidants, there is urgent need to discover novel agents from natural sources. Therefore a study was curated on a versatile medicinal plant *Tephrosia purpurea* (L.) Pers.

Tephrosia purpurea (L.) Pers. (T.P) is a highly acclaimed medicinal plant of the Ayurveda belonging to the family Fabaceae. It is an annual, perennial shrub having pantropical distribution. The plant is commonly known as 'Sarwawranvishapaka' as quoted in the Ayurvedic system of medicine due to its wound healing potential (Deshpande *et al*., 2003). The plant is known to have immense pharmacological significance as studies have reported anticarcinogenic, antimicrobial, hepatoprotective, antidiarrheal, diuretic, and insecticidal activities from different parts of the plant (Rao *et al*., 2020). Traditionally the plant is used for the treatment of impotency, asthma, hemorrhoids, rheumatism, and enlargement of spleen and liver. Phytochemical studies have revealed the presence of rotenoids, glycosides, sterols, flavanols, isoflavones, tannins, and phenols which provide therapeutic properties (Katakam *et al*., 2019). Keeping in view the traditional and therapeutic potential of this plant, the present study was curated to evaluate the antioxidant, antimicrobial and phytochemical quantification of the extracts of *T. purpurea* to exploit its therapeutic utilities rendered in combating diseases caused by resistant micro-organisms and oxidative stress.

Materials and Methods

Plant material

The vegetative aerial parts of the plant were collected from Kund hills (28.0614°N, 76.5305°E) (Rewari district) of Aravalli hill ranges of Haryana, India. The plant samples were identified by the experts of the Department of Botany, M.D.U, Rohtak and a voucher specimen V.S.No. Rao: 101 was deposited in the Dept. of Botany, M.D.U, Rohtak for future reference. The samples were thoroughly washed with running tap and rinsed with distilled water and then dried in shade. The dried samples were grinded in a mixer-grinder and sieved through a 60 mm sieve. The dried powder was stored in Amber glass bottles in a moisture free zone.

Reagents and standards

Ethanol, Methanol, Chloroform, Whatman filter paper, Mayer's solution, Wagner's solution, Benedict's solution, sulphuric acid, acetic anhydride, ninhydrin, n-butanol, sodium hydroxide, pyridine, hydrochloric acid, sodium nitroprusside, ferric chloride, lead acetate, 2,22-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), agar, Nutrient and potato dextrose broth, formic acid, acetonitrile were procured from sigma-Aldrich (Germany). The other chemicals and reagents used in the study were of analytical grade available commercially.

Microorganism and culture conditions

The microorganisms used to assess the antimicrobial activity of the plant extracts were obtained from laboratory of enzymology recombinant DNA technology, Dept. of Microbiology, M.D.U, Rohtak. The Gram positive bacterial strains viz. *Bacillus subtili* (MTCC 1133), *Staphylococcus aureus* (MTCC 3160) and Gram negative, *Escherichia coli* (MTCC 1572), *Pseudomonas aeruginosa* (MTCC 2581) were used and maintained on nutrient agar plate at 4°C. Antifungal activity was assessed by using fungus *Aspergillus oryzae* (KC914095) and *Fusarium oxysporum* (MTCC-7392). The microorganisms were maintained on PDA plate at 4 °C as described by Saini *et al*. (2022).

Preparation of extracts by Soxhlet extraction

The plant extracts were made utilizing the Soxhlet extraction method described earlier by Okeke *et al*. (2001). The plant powder was extracted separately with different solvents *viz*. ethanol and methanol. Plant powder to solvent ratio was kept at 1:5. Powder was packed in extraction thimble made of Whatman filter paper and placed in the extraction chamber which was suspended above the solvent

flask. The extraction was carried at 60-80°C for 48 hrs and till the dark color appears in the Solvent. The extract obtained was subjected to lyophilization to remove excess solvent using Martin Christ ALPHA 1-2 LD plus D-37520 apparatus. The crude extracts *Tephrosia purpurea* Methanol extract (TPME) and *Tephrosia purpurea* Ethanol extract (TPEE) obtained were stored at 4°C in a dark place for further analysis.

The % Yield of the extracts was calculated using the following equation:

$$\% \ Yield = (EW \times 100) \div PW$$

Where, EW is the weight of crude extract and PW is the weight of the dry powder of plant samples.

Phytochemical analysis

Preliminary screening of various phytochemicals present in crude extracts of TPME and TPEE was performed as described previously by Tomar and Kaushik (2014) and Sharma *et al*. (2020). The qualitative presence of alkaloids, carbohydrates, sterols, terpenoids, triterpenoids, saponin, flavonoids, proteins, glycoside, tannins and phenols was detected.

Determination of Antioxidant activity

DPPH assay

The antioxidant potential and ability to release hydrogen atom of TPME and TPEE were assessed as described previously by Mensor *et al*. (2001). Crude extracts of TPME and TPEE were dissolved in methanol and were serially diluted with 0.3M, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) diluted with methanol, and the solution was mixed gently and incubated for 30 min at 25°C in the dark. The free radicle scavenging activity was measured at 517nm against a blank containing DPPH only. Percent inhibition of free radicle was calculated using the below mentioned formula.

$$\% \text{ free radical scavenging activity} = (AbsC - AbsS) \div AbsC \times 100$$

Where, AbsCis the absorbance of the control sample and AbsSis the absorbance of the sample.

ABTS assay

Reactive oxygen species scavenging activity of TPME and TPEE was determined by ABTS assay as described by Shirwaikar *et al*. (2006). An aqueous solution of 7mM ABTS was mixed with 2.4mM of potassium persulphate in the dark room and incubated at 25°C for 12-15 hrs. in the dark, till the occurrence of intense blue color. Afterward, 10-15µl of dissolved TPME and TPEE was mixed with 200 µl of activated ABTS solution followed by 30 min incubation at room temperature. Decolorization of the ABTS cation was measured at 734nm. Free radicle inhibition of extracts was calculated using the following formula:

$$\% \text{ free radical scavenging activity} = (AbsC - AbsS) \div AbsC \times 100$$

where, AbsCis the absorbance of the control sample and AbsSis the absorbance of the sample.

IC_{50} value

Half maximal inhibitory concentration ($IC_{50,}$ µg/ml) was calculated by plotting a graph of percent inhibition against the concentration of crude extracts. The IC_{50} value of plant extract is defined as the concentration which quenches 50 % free radical produced by DPPH or ABTS.

Determination of Antimicrobial activity

Antimicrobial activity

The bacterial strains were precultured in nutrient broth (NB) overnight at 37 °C at turbidity of 0.5 McFarland (10^8 CFU/mL), and fungal inoculums were prepared in potato dextrose broth (PDB) after incubation of 72 hrs. Agar disc diffusion method following Pandey *et al*. (2011) was used to assess the antibacterial and antifungal properties of the extracts. Afterward, 20 µl of bacterial strain was spread on petri plate poured with nutrient agar (for antibacterial), potato dextrose agar (for antifungal) and sterile Whatman discs were impregnated with 30 µl of serially diluted concentrations of TPME & TPEE (100, 50, 25, 12.5 & 6.25 mg/ml). Plates were incubated at 37°C for 24 hrs. (for antibacterial) and 72 hrs (for antifungal) to achieve a clear zone of inhibition, and was measured using scale. Cefotaxime and fluconazol were used as positive control for antibacterial and antifungal activity respectively. DMSO was used as negative control.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of TPME and TPEE was determined against four bacterial strains using Resazurin dye microdilution method (Sarker *et al*., 2007). 96 well sterile microplates were labeled and 100 µl of TPME & TPEE were pipetted in the first row and 50 µl of NB

was poured in all the wells. Serial dilution using multichannel pipette was performed and 15 µl of bacterial strains was poured in all the wells. 10 µl of Resazurin dye was poured in all the wells before incubating for 24 hrs. at 37 °C. The change in color from dark blue to pink depicted the growth of bacteria, and colorimetric estimation was carried to estimate the MIC. Positive control and negative control were also kept for reference.

Quantitative Screening of Plant Extract

FTIR analysis

To identify the presence of functional groups of compounds in crude extracts of TPME and TPEE were subjected to fourier transform infrared spectrophotometer (FTIR), Perkin Elmer Spectrum IR version 10.62. Briefly, 10-15 mg of each crude extracts were entrapped in 150-200 mg of potassium bromide in a palatable form. The FTIR spectroscope scan was set with a range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. IR spectra were compared with FTIR database.

LC-MS/MS analysis

Crude extracts of TPME and TPEE was subjected to Agilent 6470 LC-MS equipped with ZORBAX C18 column (2.1 mm × 2.3 x 100 mm) for LC-MS analysis with positive mode of electron spray ionization (ESI). Water (A) and acetonitrile (B) having 1 % formic acid were used as gradient elution system. The gradient was started at 5 % B and maintained for 2 min at a flow rate of 0.2 mL/min, followed by a linear increase to 90 % B at the same flow rate from 2 to 34 min, then maintained for 2 min at 90 % B. Mass spectrometer was optimized with positive polarity mode having following parameters: 380 °C gas temperature, nebulizer 50 Psi, 3500V capillary voltage, 500V nozzle voltage, flow rate 12 ml m^{-1}. The injection volume of the extract was set at 10 µL.

Statistical Analysis

All the experiments were performed in triplicates and the data was expressed as mean ± SD.

Results and Discussion

The present study revealed and compared the potential of *T. purpurea* methanol and ethanol extracts for antioxidant, antimicrobial, and phytochemical assessment. The % yield of the plant extracts was calculated using the weight of the crude extract obtained after soxhlet extraction and the amount of plant powder used for the extraction process. The per cent yield of the plant extracts (TPME and TPEE) was 23.7 and 22.16, respectively. It was evident from the findings of the study that vegetative aerial parts of the plant harbor plethora of biologically active phytocompounds amongst which majority of them extracted in polar solvents like methanol and ethanol as was described earlier by Patial *et al*. (2019); Chaqroune and Taleb (2022).

The qualitative phytochemical analysis of the T.P plant extracts indicated the presence of proteins, carbohydrates, saponins, flavonoids, glycosides, tannins and phenolic compounds in both the extracts (TPME and TPEE). However, the TPEE indicated the presence of alkaloids and lacked sterols and triterpenoids the results were in consensus with the findings of Gopalakrishnan *et al*. (2009); Khan *et al*. (2017); Sharma *et al*. (2020). The phytochemical richness in the plant extracts could be attributed to the leaves of the plants which are known to have numerous compounds and the solvent systems which are used for the extraction process (Altemimi *et al*. 2017). Kuppusamy *et al*. (2015) reported polar solvents are known to extract flavonoid, phenolic, glycosides and steroids from plants.

The free radical scavenging activity of both the plant extracts was assessed against ABTS and DPPH radicals. The antioxidant compounds present in the plant extracts react with free radical DPPH and convert it to 1, 1-diphenyl-2-picrrylhydrazine by donation of electron or hydrogen atom (Thambiraj *et al*. 2012; Egharevba *et al*., 2019). Similarly, the plant extracts reduce ABTS oxidants. Ascorbic acid was used as standard in both the assays and showed IC_{50} value of 11.5 and 36.54 µg/ml against ABTS and DPPH respectively. The TPEE and TPME scavenged DPPH with IC_{50} value of 78.7 and 65.38 µg/ml respectively. The ABTS scavenging IC_{50} values of TPEE and TPME were found to be 49.7 and 55.38 µg/ml respectively. The rich antioxidant potential of the plant extracts has been dedicated to the presence of flavonoids and polyphenols by many researchers (Khan *et al*., 2017; Patel *et al*., 2010; Nile *et al*., 2012). The TPME was more active than TPEE primarily due to presence of high total flavonoid content (TFC) as was suggested by Padmapriya *et al*. (2017) and Jain *et al*. (2013). It is also noteworthy that flavonoids and polyphenolic compounds accumulate more in the aerial parts of the plant (leaves) to ren-

der protection against predators and hence, aerial parts of the plant intend to show higher antioxidant activities (Manach *et al*., 2004; Sreenivasan and Subburaju, 2012).

The radical scavenging activity of plant extracts and Ascorbic acid against ABTS & DPPH have been depicted in Figure 1 (a) and (b). Table 1 and 2 depicts the radicle scavenging activity of plant extracts and Ascorbic acid against DPPH and ABTS radicals.

Antimicrobial activity of T.P extracts was evaluated by measuring zone of inhibition (ZOI) obtained. The antibiotic cefotaxime considerably inhibited all the bacterial strains with ZOI ranging from 26.6±0.2 to 28.3±1.5 for *S. aureus* and *P. aeuroginosa.* The TPEE showed concentration dependent inhibition of bacterial growth against all the bacterial strains with highest inhibition was observed on *S. aureus* (18.3±0.5) followed by *B. subtilis* (18±1.1), *P. aeuroginosa* (17±1), and least inhibition of *E. coli* (13.3±0.5) at 100mg/ml. However, TPEE strongly inhibited fungus *F. oxysporum* (14±1) as compared to standard fluconazol (17.6±0.5). Similarly, the TPME extract strongly inhibited *S. aureus* (15.3±0.5) ^ *B. subtilis* (14.6±0.5) ^ *P. aeuroginosa* (12.3±0.5) ^ *E. coli* (11.6±1.5), and *A. oryzae* (12.6±0.5) ^ *F. oxysporum* (11.6±0.5). The MIC of TPEE was found to be similar for all the bacterial strains (3.125), and lowest against fungus *F. oxysporum* (12.5). Similarly, TPME exhibited similar inhibition pattern against all the bacterial strains with ZOI ranging from 11.6±1.5 to 15.3±0.5. The MIC of TPME against bacterial strains was ranging from 1.62 to 3.125 and 6.25, 12.5 mm for fungal strains *F. oxysporum* and *A. oryzae* respectively. It was also evident from the results that Gram positive bacteria were more susceptible than Gram negative bacteria towards the plant extracts as has been reported in previous findings (Egharevba *et al*., 2019; Manikandan *et al*., 2014; Kumar *et al*., 2019; Andhare and Shinde, 2020). The antibacterial activity by both the extracts could be deduced due to

Table 1. Free radical scavenging activity of *T. purpurea* extracts against DPPH radicals.

Conc. (µg/ml)	RSA of Ascorbic acid	TPME	TPEE
10	29.6±1.0	18.9±0.6	17.3±0.5
20	36.0±0.4	22.1±0.7	19.1±0.8
30	48.6±1.2	28.8±0.3	23.8±1.0
40	53.3±0.8	33.2±0.6	29.3±0.7
50	58.6±0.6	38.6±0.4	34.6±0.9
60	67.0±0.9	45.2±0.4	39.3±1.3
70	76.5±0.4	52.2±0.7	44.4±1.3
80	81.6±0.5	59.4±0.5	49.7±0.2
90	85.7±01	65.7±1.2	56.6±0.3
100	88.4±01	72.6±1.5	63.1±0.8

Table 2. Radical scavenging activity of *T. purpurea* extracts against ABTS radicals

Conc. (µg/ml)	RSA of Ascorbic acid	TPME	TPEE
10	46.8±0.9	29.4±0.5	32.3±1.2
20	52.7±0.8	32.6±1.3	36.3±0.7
30	59.4±0.2	35.5±0.7	39.5±0.9
40	67.8±0.7	40.0±0.9	43.6±0.4
50	71.4±1.1	44.6±1.4	48.9±0.6
60	76.5±0.4	49.6±0.2	51.6±0.8
70	80.7±0.9	56.8±0.5	58.4±1.4
80	84.3±0.8	63.4±1.0	66.0±0.2
90	87.5±0.7	69.7±0.8	71.6±0.9
100	94.6±01	76.4±0.8	78.8±0.7

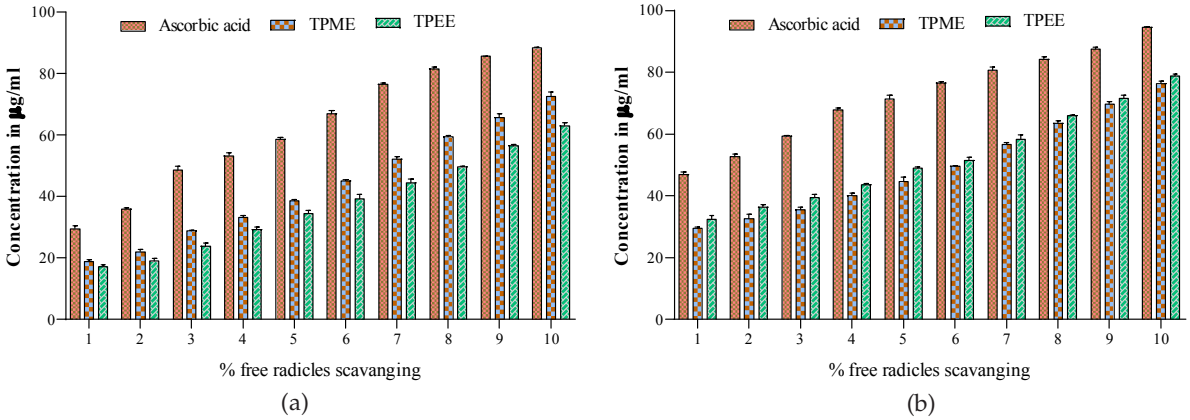


Fig. 1. (a)DPPH radical scavenging activity of *T. purpurea* extracts and ascorbic acid(b)ABTS radical scavenging activity of *T. purpurea* extracts and ascorbic acid

higher extracting potential of antimicrobial agents in alcoholic solvents (Rangama *et al*., 2009). The plant extracts were found to inhibit fungus species at higher concentrations only (100, 50 and 25 mg/ml) and were susceptible to TPME more than TPEE. Concordant findings have also been reported for *Aspergillus sp.* (Naik *et al*., 2013). The mean ZOI obtained against bacterial and fungal species have been depicted in Figure 2 and Table 3 and MIC of both the plant extracts in Table 4.

TPEE and TPME were subjected to FT-IR analysis for quantification of the functional groups present at prominent peaks. The TPEE and TPME showed similar absorption band patterns except for peak values at 1365, 1273 and 809 cm^{-1} which were seen in TPEE and these peaks attributed to C-N Stretching (aromatic), C-N Stretching (primary and secondary aromatic amines), and C=C Bending (alkene), respectively. While, the absorption band at 3435 cm^{-1} represented the O-H stretching (alcohols/phenols), 2918, 2850 cm^{-1} attributed O-H and C-H stretching (carboxylic acid/ alkanes), respectively. The peaks at 1633, 1510 and 1462 cm^{-1} represented the presence of N-H bend (1° amines), N-O Stretching (N-monosaturated amides), and C-H Bend (alkanes/ *gem*-dimethyl), respectively. The peaks at 1203, 933, 898 and 595 cm^{-1} corresponding to C-N Stretching (aliphatic amines), O-H bend (carboxylic acid), C-H bending (1,2,4-trisubstituted/aromatics), C-Br stretching (alkyl halides), and showed high transmittance. Similar findings were also reported by Kumar *et al*. (2019); Karunanithi and Chellappa

Table 3. Antimicrobial activity of *T. purpurea* extracts

S. No.	Solvents	Microbial culture	ZOI (mm) in different Conc. (mg/ml) of different extracts					
			100	50	25	12.5	6.25	Positive control
1.	Ethanol	*S. aureus*	18.3±0.5	16.6±1.1	14.6±0.5	13.3±0.5	12.6±0.5	26.6±0.2
		E. coli	13.3±0.5	11.6±0.5	10.3±0.5	9.3±2.3	8.6±1.1	28.0±1.0
		B. subtilis	18.0±1.1	16.6±0.5	14.6±0.5	14.3±0.5	11.6±1.0	27.6±1.1
		P. aeuroginosa	17.0±1.0	15.3±0.5	14.3±0.5	12.6±0.4	9.6±0.4	28.3±1.5
		A. oryzae	11.6±1.5	10.6±0.5	9.0±1.0	0	0	17.0±1.0
		F. oxysporum	14.0±1.0	13.6±1.5	9.3±0.5	0	0	17.6±0.5
2.	Methanol	*S. aureus*	15.3±0.5	13.6±0.5	12.0±0.6	10.6±1.1	9.3±0.5	26.6±2.5
		E. coli	11.6±1.5	10.0±1.0	9.6±0.5	7.6±0.5	7.6±0.5	28.3±1.1
		B. subtilis	14.6±0.5	14.0±0.6	12.6±0.5	11.6±0.5	10.6±1.1	28.3±1.5
		P. aeuroginosa	12.3±0.5	10.6±0.5	9.6±1.0	10.0±0.5	9.0±1.0	28.6±1.5
		A. oryzae	12.6±0.5	11.3±1.1	9.0±1.0	0	0	17.6±1.1
		F. oxysporum	11.6±0.5	10.3±0.5	10.0±1.0	0	0	18.0±1.0

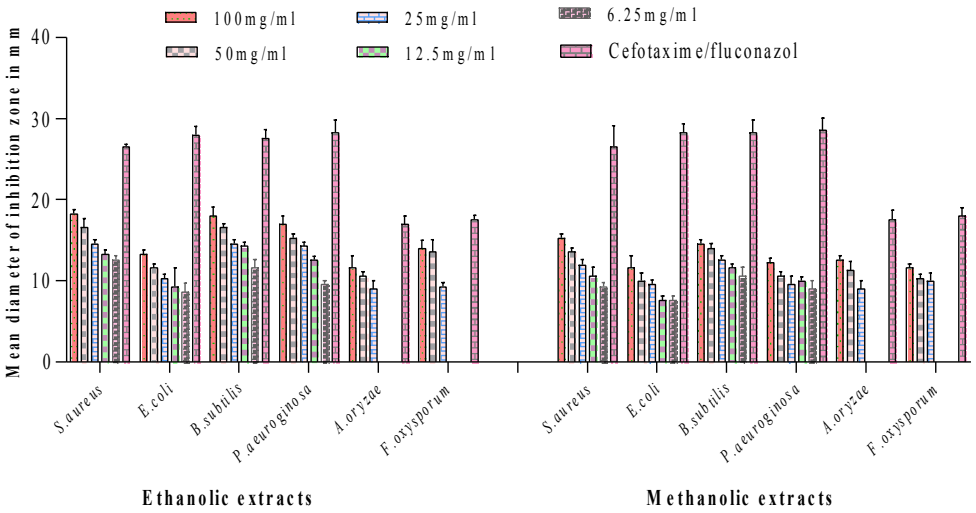


Fig. 2. Mean Zone of inhibition against different microbial populations

(2019); Jain *et al*. (2013). Figure 3 represents the FTIR spectrum of the plant extracts.

The LC-MS/MS quantification of TPEE and TPME revealed the presence of numerous Phytocompounds which have been summarized in the Table 5 along with the Retention time (RT), Molecular formula, weight, % area and their presence in respective extracts. Figure 4 depicts the LC-MS/MS chromatogram with m/z breakup for identified compounds. Total 37 compounds were identified from both the extracts with major presence of N-(3-Amino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-N-phenylacetamide (10.85 %); 9,12,15-Octadecatrienoic acid, methyl,ester, (z,z,z)- (1.92%); Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (2.05%); Hexadecanoic acid (3.21%) in TPEE and Stigmasterol (5.34%); 2-Methoxy-4-vinylphenol (0.76%); 3-Azetidin-1-yl-propionic acid, methyl ester (3.84%); Di(pentamethylphenyl)ketone (19.88%); D-Glucopyranosiduronic acid, 3-(5-ethylhexahydro-1,3-dimethyl-2,4,6-Trioxo-5-primidinyl)-1-Methylbutyl 2,3,4-Tris-O-(Trimethylsilyl)-Methyl ester (4.13%) in TPME. While, 3b, 4, 5,6, 7, 7a, 9, 10, 11, 12-Decahydrobenzo[b] fluoranthene (2.12, 10.18%) and Phytol (4.41, 7.99%) were present in both the extracts TPEE and TPME in their respective % area. Karunanithi and Chellappa (2019) and Kumar *et al*. (2019) had earlier identified 2-Methoxy-4-vinylphenol; Hexadecanoic acid; Phytol; 9,12,15-Octadecatrienoic acid, methyl, ester,(z,z,z)-; Stigmasterol; 9, 12, 15-Octadecatrienoic acid and Hexadecanoic acid, methyl esterusing GC-MS and GC-HRMS.

However, few studies have been conducted to screen the T.P extracts and the considerable presence of phytocompounds like Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester; 3-Azetidin-1-yl-propi-

Table 4. Minimum Inhibitory Concentration of *T. purpurea* extracts

S. No.	Solvents	Microbial culture	Minimum Inhibitory Concentration (MIC)
1.	Ethanol	*S. aureus*	3.125
		E. coli	3.125
		B. subtilis	3.125
		P. aeuroginosa	3.125
		A. oryzae	25
		F. oxysporum	12.5
2.	Methanol	*S. aureus*	1.62
		E. coli	3.125
		B. subtilis	1.62
		P. aeuroginosa	3.125
		A. oryzae	12.5
		F. oxysporum	6.25

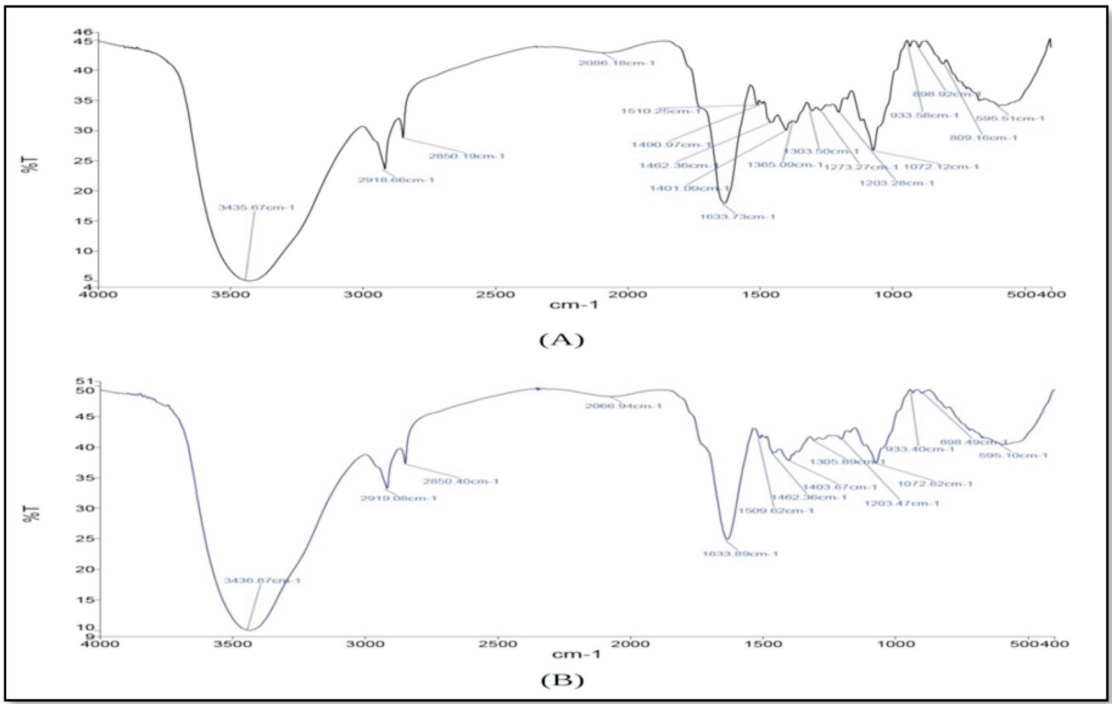


Fig. 3. FT-IR Spectrum of TPEE (A) and TPME (B)

Table 5. Major Compounds identified from TPEE and TPME by LC-MS

S. No.	Identified Compound	RT (min)	Molecular formula	M.wt (g/mol)	% Area	Extract
1.	Phytol	8.247	$C_{20}H_{40}O$	296.5	4.41	TPEE
2.	Hexadecanoic acid	7.717	$C_{18}H_{36}O_2$	284.5	3.21	TPEE
3.	2-(tert.-Butyldimethylsilyl)oxybenzylideneacetophenone	2.902	$C_{21}H_{26}O_2Si$	338.5	0.35	TPEE
4.	9, 12, 15-Octadecatrienoic acid	8.482	$C_{27}H_{52}O_4Si_2$	469.9	1.84	TPEE
5.	9,12,15-Octadecatrienoic acid, methyl,ester,(z,z,z)-	8.198	$C_{19}H_{32}O_2$	292.5	1.92	TPME
6.	Heptadecanoic acid, 15-methyl-, ethyl ester	8.548	$C_{20}H_{40}O_2$	312.5	1.13	TPEE
7.	1-Hexadecanol, 2-methyl-	5.115	$C_{17}H_{36}O$	256.5	0.62	TPEE
8.	2-Pentadecanone, 6,10,14-trimethyl	7.156	$C_{18}H_{36}O$	268.5		TPEE
9.	Ethanamine, N,N-diethyl-2,2-dimethyl-2-[(3,5-dimethoxy) phenyl]	7.512	$C_{16}H_{27}NO_2$	265.39	0.80	TPEE
10.	Cis-13-Eicosenoic acid	6.925	$C_{20}H_{38}O_2$	310.5	0.45	TPEE
11.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	15.750	$C_{16}H_{48}O_7Si8$	577.2	2.05	TPEE
12.	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	12.512	$C_{30}H_{52}O$	428.7	1.36	TPEE
13.	Propanoic acid-3-oxo, 3-(2,4-dichlorophenyl)-ethyl ester	6.586	$C_{11}H_{10}C_{l2}O_3$	261.1	0.53	TPEE
14.	N-(3-Amino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-N-phenylacetamide	11.098	$C_{18}H_{14}N_2O_3$	306.3	10.85	TPEE
15.	3b,4,5,6,7,7a,9,10,11,12-Decahydrobenzo[b]fluoranthene	11.416	$C_{20}H_{22}$	262.4	2.12	TPEE
16.	2-Methoxy-4-vinylphenol	4.8	$C_9H_{10}O_2$	150.17	0.76	TPME
17.	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	12.508	$C_{30}H_{52}O$	428.7	1.73	TPME
18.	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-methyl ester	7.123	$C_{21}H_{38}O_2$	322.5	0.46	TPME
19.	1-Heptatriacotanol	8.921	$C_{37}H_{76}O$	537	0.46	TPME
20.	(BORONAL) 2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	6.474	$C_{14}H_{22}O$	206.32	0.60	TPME
21.	4,7-Dimethoxy-2-methyl-1H-indene	6.19	$C_{12}H_{14}O_2$	190.24	0.60	TPME
22.	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	7.026	$C_{11}H_{16}O_3$	196.24	0.70	TPME
23.	Octaethylene glycol monododecyl ether	7.155	$C_{28}H_{58}O_9$	538.8	0.81	TPME
24.	2,6,10,10-tetramethyl-1-oxaspiro[4.5]decan-6-ol	6.588	$C_{13}H_{24}O_2$	212.33	0.88	TPME
25.	5-Methyl-2-[5-(2-methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]- Phenol	12.279	$C_{18}H_{15}N_3O_2$	305.3	1.29	TPME
26.	3-Azetidin-1-yl-propionic acid, methyl ester	3.744	$C_7H_{13}NO_2$	143.18	3.84	TPME
27.	1-Naphthol, 1,2,3,4-tetrahydro-2-methyl-	4.208	$C_{11}H_{14}O$	162.23	1.47	TPME
28.	Vitamin E	15.235	$C_{29}H_{50}O_2$	430.7	1.73	TPME
29.	Phorbol	12.378	$C_{20}H_{28}O_6$	364.4	0.70	TPME
30.	3b,4,5,6,7,7a,9,10,11,12-Decahydrobenzo[b] fluoranthene	11.431	$C_{20}H_{22}$	262.4	10.18	TPME
31.	Di(pentamethylphenyl)ketone	10.885	$C_{23}H_{30}O$	322.5	19.88	TPME
32.	D-Glucopyranosiduronic acid, 3-(5-ethylhexahydro-1,3-dimethyl-2,4,6-Trioxo-5-primidinyl)-1-Methylbutyl 2,3,4-Tris-O-(Trimethylsilyl)-,Methyl ester	15.765	$C_{29}H_{56}N_2O_{10}Si_3$	677	4.13	TPME
33.	Stigmasterol	17.007	$C_{29}H_{48}O$	412.7	5.34	TPME
34.	Hexadecanoic acid, methyl ester	7.452	$C_{17}H_{34}O_2$	270.5	18.38	TPME
35.	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	7.294	$C_{19}H_{34}O_6$	358.5	6.18	TPME
36.	Phytol	8.248	$C_{20}H_{40}O$	296.5	7.99	TPME
37.	Hexa-t-butylselenatrisiletane	10.643	$C_{24}H_{54}SeSi_3$	505.9	1.09	TPME

onic acid, methyl ester; 5-Methyl-2-[5-(2-methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]- Phenol; 3b,4,5,6,7,7a,9,10,11,12-Decahydrobenzo[b] fluoranthene and N-(3-Amino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-N-phenylacetamide were identified for the first time from the plant extracts of T.P. The variations in the compounds identified from the T.P could be due to the difference in the

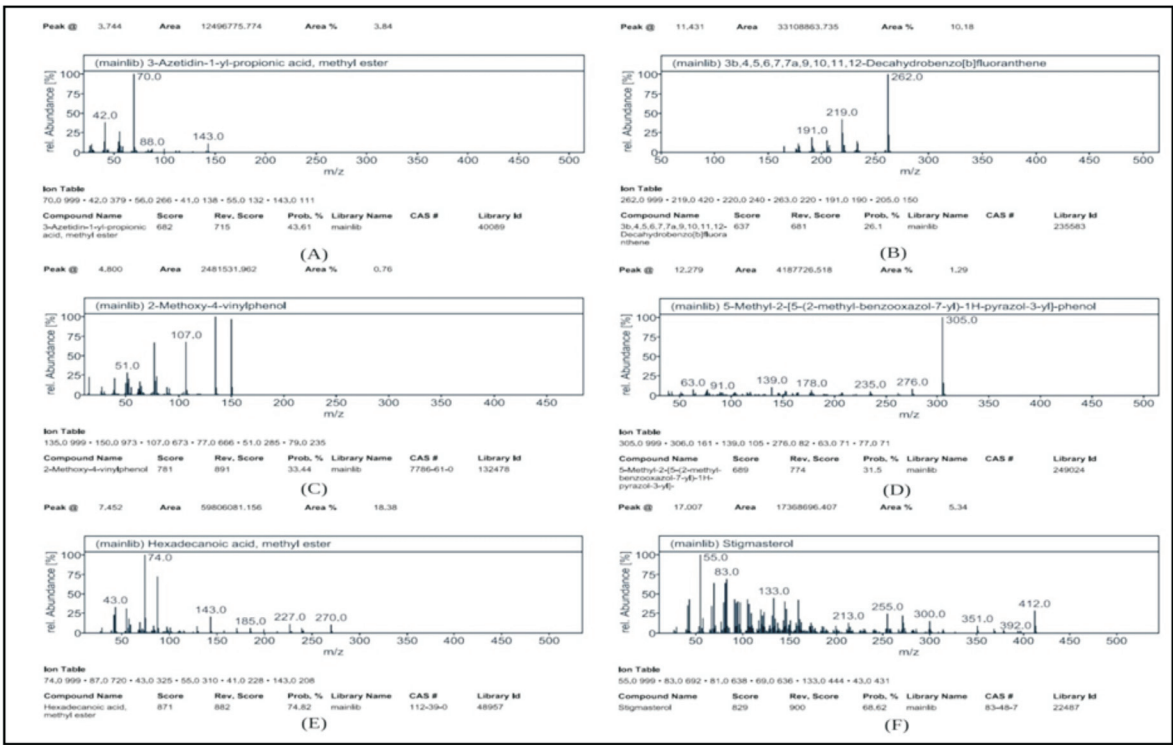
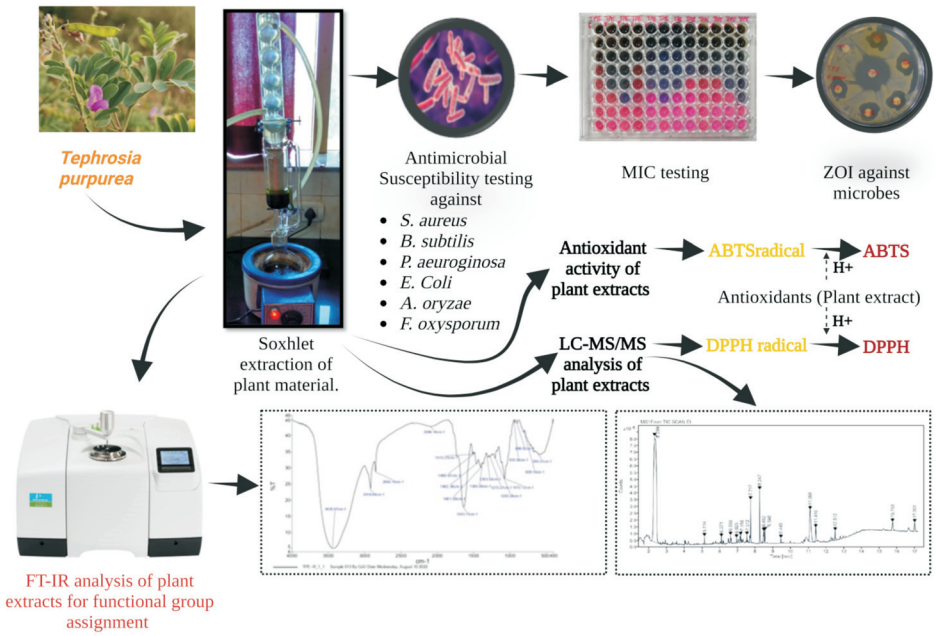


Fig. 4. LC-MS characterization of (A) 3-Azetidin-1-yl-propionic acid, methyl ester, (B) 3b,4,5,6,7,7a,9,10,11,12-Decahydrobenzo[b]fluoranthene, (C) 2-Methoxy-4-vinylphenol (D) 5-Methyl-2-[5-(2-methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]- Phenol, (E) Hexadecanoic acid, methyl ester, (F) Stigmasterol



techniques utilized, column used and variability in the mobile and stationary phase for differentiation of the compounds (Kuehnbaum and Britz-McKibbin 2013; Pintea and Magdas, 2022). The presence of 2-Methoxy-4-vinylphenol (Alghamdi *et al*., 2018; Jeong and Jeong, 2010), Phytol (Santos *et al*., 2013;Okpala *et al*., 2022), Stigmasterol (Yabalak *et al*., 2022) and 5-Methyl-2-[5-(2-methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]- Phenol justified the antioxidant, and antimicrobial potential of the plant extracts.

Conclusion

The extracts of *T. purpurea* confirmed the presence of carbohydrates, proteins, saponins, flavonoids, glycosides, phytosterols, tannins and phenolic compounds. The TPME and TPEE showed considerable antioxidant potential chiefly owning to the presence of diverse array of phytocompounds specifically 2-Methoxy-4-vinylphenol, Phytol, Stigmasterol and 5-Methyl-2-[5-(2-methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]- Phenol which were also confirmed by LC-MS quantification. The plant showed potent antimicrobial activity indicating the presence of novel antimicrobial compounds in the plant. The LC-MS/MS based quantification of the plant further revealed the probability of presence of novel phytocompounds which could be responsible for the therapeutic efficacy of the plant. Thus, it is concluded that studied plant is a potential source of natural antioxidant and antimicrobial agents. It has significant phytotherapeutic potential, and future research towards isolation and pharmaceutical applications should be considered.

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Conflict of Interest

The authors have no conflicts of interest to declare.

References

Alghamdi, S.S., Khan, M.A., El-Harty, E.H., Ammar, M.H., Farooq, M. and Migdadi, H.M. 2018. Comparative phytochemical profiling of different soybean (*Glycine max* (L.) Merr) genotypes using GC–MS. *Saudi Journal of Biological Sciences*. 25(1): 15-21.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G. and Lightfoot, D.A. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*. 6(4): 42.https://doi.org/10.3390/plants6040042.

Andhare, A.A. and Shinde, R.S. 2020. In vitro Studies on Antimicrobial Sensitivity of *Staphylococcus aureus* from *Prosopisjuliflora*, *Cassia occidentalis* and *Tephrosia purpurea*. *Anti-Infective Agents*. 18(4): 384-390.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E.M., Linder, T., Wawrosch, C., Uhrin, P. and Stuppner, H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*. 33(8): 1582-1614.

Bhuvaneswari, T.K. 2020. Corrosion inhibition of mild steel in hydrochloric acid by leaves extract of *Tephrosia purpurea*. *Journal of Adhesion Science and Technology*. 34(22): 2424-2447.

Chaqroune, A. and Taleb, M. 2022. Effects of Extraction Technique and Solvent on Phytochemicals, Antioxidant, and Antimicrobial Activities of Cultivated and Wild Rosemary (*Rosmarinus officinalis* L.) from Taounate Region. *Biointerface Res. Appl. Chem*. 12: 8441-8452.

Chopra, B. and Dhingra, A.K. 2021. Natural products: A lead for drug discovery and development. *Phytotherapy Research.* 35(9): 4660-4702.

Choudhary, G.P. 2007. In vitro antioxidant studies of the ethanolic extract of *Tephrosia purpurea* L. *Ancient Science of Life*. 27(1): 26-30.

Deshpande, S.S., Shah, G.B. and Parmar, N.S. 2003. Antiulcer activity of *Tephrosia purpurea* in rats. *Indian journal of Pharmacology*. 35(3): 168-172.

Egharevba, G.O., Dosumu, O.O., Oguntoye, S.O., Njinga, N.S., Dahunsi, S.O., Hamid, A.A. and Priyanka, U. 2019. Antidiabetic, antioxidant and antimicrobial activities of extracts of *Tephrosia bracteolata* leaves. *Heliyon*. 5(8): e02275.

Gopalakrishnan, S., Vadivel, E. and Dhanalakshmi, K. 2009. Phytochemical and pharmacognostical studies of *Tephrosia purpurea* Linn., aerial and root parts. *J. Herb Med Toxicol*. 3(2): 73-78.

Jain, A., Nahata, A. and Singhai, A.K. 2013. Effect of *Tephrosia purpurea* (L.) pers. Leaves on gentamicin-induced nephrotoxicity in rats. *Scientia Pharmaceutica*. 81(4): 1071-1088.

Jeong, J.B. and Jeong, H.J. 2010. 2-Methoxy-4-vinylphenol can induce cell cycle arrest by blocking the hyperphosphorylation of retinoblastoma protein in benzo [a] pyrene-treated NIH3T3 cells. *Biochemical and Biophysical Research Communications.* 400(4): 752-757.

Karunanithi, B.T. and Chellappa, J. 2019. Adsorption and

inhibition properties of *Tephrosia Purpurea* as corrosion inhibitor for mild steel in sulphuric acid solution. *Journal of Dispersion Science and Technology*. 40(10): 1441-1450.

Katakam, S., Sharma, P., Anandjiwala, S., Sharma, S. and Shrivastava, N. 2019. Investigation on apposite chemical marker for quality control of *Tephrosia purpurea* (L.) Pers. by means of HPTLC-chemometric analysis. *Journal of Chromatography B*. 1110(1111): 81-86.

Khan, L.U., Khan, R.A., Ahmed, M., Khan, W.U. and Khan, M.W. 2017. Phytochemical investigation and pharmacological activities of *Tephrosia purpurea* (L.) Pers. Bark. *Int. J. Biol. Biotech.* 14(2): 173-178.

Kýlýç, C., Can, Z.,Yýlmaz, A., Yýldýz, S. and Turna, H. 2017. Antioxidant Properties of Some Herbal Teas (Green tea, Senna, Corn Silk, Rosemary) Brewed at Different Temperatures. *International Journal of Secondary Metabolite*. 4(3, Special Issue 1): 142-148.

Kuehnbaum, N.L. and Britz-McKibbin, P. 2013. New advances in separation science for metabolomics: resolving chemical diversity in a post-genomic era. *Chemical Reviews*. 113(4): 2437-2468.

Kumar, V., Singh, S., Singh, A., Dixit, A.K., Srivastava, B., Sidhu, G.K. and Prakash, O. 2018. Phytochemical, antioxidant, antimicrobial, and protein binding qualities of hydro-ethanolic extract of *Tinosporacordifolia*. *Journal of Biologically Active Products from Nature*. 8(3): 192-200.

Kumar, V., Singh, S., Srivastava, B., Patial, P.K., Kondalkar, S.A. and Bharthi, V. 2019. Volatile and semi-volatile compounds of *Tephrosia purpurea* and its medicinal activities: experimental and computational studies. *Biocatalysis and Agricultural Biotechnology*. 20: 101222.

Kuppusamy, P., Yusoff, M.M., Parine, N.R. and Govindan, N. 2015. Evaluation of in-vitro antioxidant and antibacterial properties of *Commelinanudiflora* L. extracts prepared by different polar solvents. *Saudi Journal of Biological Sciences*. 22(3): 293-301.

Manach. C., Scalbert, A., Morand, C., Rémésy, C. and Jiménez, L. 2004. Polyphenols: food sources and bioavailability. *The American Journal of Clinical Butrition*. 79(5): 727-747.

Manikandan, B., Perumal, R., Vijayakumar, P., Dhayalkarthick, N., Selvamaleeswaran, P. and Sureshkumar, M. 2014. Antimicrobial activity of medicinally important plant-*Tephrosia purpurea* Linn. against pathogenic bacteria. *Journal of Chemical and Pharmaceutical Research*. 6(9): 61-64.

Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C.D., Coube, C.S. and Leitão, S.G. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*. 15(2): 127-130.

Naik, A.L.J., Reddy, S. and Rayalu, D.J. 2013. Phytochemical analysis, TLC profiling and antimicrobial activity of *Tephrosia purpurea*. *Int. J. of Pharm. & Life Sci.* 4(2): 2375-2379.

Nile, S.H., Khobragade, C.N. and Park, S.W. 2012. Antioxidant, Anti-inflammatory and Xanthine oxidase inhibitory Activity of *Tephrosia purpurea* L. *International Journal of Phytomedicine*. 4(2): 266-271.

Okeke, M.I., Iroegbu, C.U., Eze, E.N., Okoli, A.S. and Esimone, C.O. 2001. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. *Journal of Ethnopharmacology*. 78(2-3): 119-127.

Okpala, E.O., Onocha, P.A. and Ali, M.S. 2022. Antioxidant activity of phytol dominated stem bark and leaf essential oils of Celtiszenkeri Engl. *Trends in Phytochemical Research*. 6(2): 137-144.

Padmapriya, R., Ashwini, S. and Raveendran, R. 2017. In vitro antioxidant and cytotoxic potential of different parts of *Tephrosia purpurea*. *Research in Pharmaceutical Sciences*. 12(1): 31-37.

Pandey, P., Mehta, A. andHajra, S. 2011. Evaluation of antimicrobial activity of *Rutagraveolens* stem extracts by disc diffusion method. *Journal of Phytology*. 3(3): 92-95.

Pasdaran, A., Delazar, A., Ayatollahi, S.A. and Pasdaran, A. 2017. Chemical composition and biological activities of methanolic extract of *Scrophularia oxysepala* Boiss. *Iranian Journal of Pharmaceutical Research*. 16(1): 338-346.

Patel, A., Patel, A. and Patel, N.M. 2010. Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* linn.leaves (Leguminosae). *Pharmacognosy Research*. 2(3): 152.

Patial, P.K., Sharma, A., Kaur, I. and Cannoo, D.S. 2019. Correlation study among the extraction techniques, phytochemicals, and antioxidant activity of Nepetaspicata aerial part. *Biocatalysis and Agricultural Biotechnology*. 20: 101275.

Pintea, A.M. and Magdas, D.A. 2022. Analytical Techniques for the Biochemical Profiling in Seabuckthorn. In The Seabuckthorn Genome. Springer, Cham. 79-111.

Prestinaci, F., Pezzotti, P. and Pantosti, A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health.* 109(7): 309-318.

Rangama, B.N.L.D., Abayasekara, C.L., Panagoda, G.J. and Senanayake, M.R.D.M. 2009. Antimicrobial activity of *Tephrosia purpurea* (Linn.) Pers. and *Mimusopselengi* (Linn.) against some clinical bacterial isolates. *Journal of the National Science Foundation of Sri Lanka*. 37(2): 139-145.

Rao, A.S., Yadav, S.S., Singh, P., Nandal, A., Singh, N., Ganaie, S.A. and Bansal, P. 2020. A comprehensive review on ethnomedicine, phytochemistry, pharmacology, and toxicity of *Tephrosia purpurea* (L.) Pers. *Phytotherapy Research*. 34(8) : 1902-1925.

Rice-Evans, C.A. and Packer, L. (Eds.). 2003. *Flavonoids in Health and Disease.* CRC Press.

Saini, S., Kumar, A., Singhal, B., Kuhad, R.C. and Sharma, K.K. 2022. Fungal oxidoreductases and CAZymes effectively degrade lignocellulosic component of switchgrass for bioethanol production. *Fuel*. 328: 125341.

Santos, C.C.D.M.P., Salvadori, M.S., Mota, V.G., Costa, L.M., de Almeida, A.A.C., de Oliveira, G.A.L. and de Almeida, R.N. 2013. Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. *Neuroscience Journal*. 2013.

Sarker, S.D., Nahar, L. and Kumarasamy, Y. 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods*. 42(4): 321-324.

Sharma, G.K., Sharma, S., Chasta, P. and Chandrul, K.K. 2020. Assessment of Pharmacognostic (Physical, Chemical and Analytical) Parameters & *In-vitro* Antioxidant Potential of *Tephrosia purpurea* (L.) Pers. *Int. J. Pharma. Sci. Rev. Res.* 65 (2): 106-113.

Shirwaikar, A., Shirwaikar, A., Rajendran, K. and Punitha, I.S.R. 2006. *In vitro* antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine. *Biological and Pharmaceutical Bulletin*. 29(9): 1906-1910.

Sreenivasan, K.K. and Subburaju, T. 2012. Comparative antioxidant studies of ethanol extract and fresh aqueous extracts of *Tephrosia purpurea*. *Pharma Science Monitor.* 3(4): 2064-2077.

Thambiraj, J., Paulsamy, S. and Sevukaperumal, R. 2012. Evaluation of *in vitro* antioxidant activity in the traditional medicinal shrub of western districts of Tamilnadu, India, *Acalyphafruticosa* Forssk. (Euphorbiaceae). *Asian Pacific Journal of Tropical Biomedicine*.2(1): 127-130.

Tomar, R.S. and Kaushik, S. 2014. In-vitro efficacy analysis of aqueous extract of *Zingiber officinale*, *Boswellia serrata* and *Coleus aromaticus* against pathogenic *Staphylococcus aureus*. *International Journal of Advanced Research.* 2(6): 51-56.

Yabalak, E., Ibrahim, F., Eliuz, E.A.E., Everest, A. and Gizir, A.M. 2022. Evaluation of chemical composition, trace element content, antioxidant and antimicrobial activities of *Verbascumpseudoholotrichum*. *Plant Biosystems*. 156(2): 313-322.