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INFLUENCE OF SOLVENT POLARITY ON PHYTOCHEMICALS EXTRACTION, ANTIOXIDANT, ENZYME INHIBITION AND ANTIMICROBIAL ACTIVITIES OF *TEUCRIUM STOCKSIANUM* BOISS

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Abstract

Teucrium stocksianum Boiss, a medicinally valuable herb, is well-reported for its anticancer, antimalarial, and analgesic properties, alongside its traditional use in treating sore throat and jaundice. Given these immense therapeutic values, it is crucial to understand how the therapeutic compounds extraction in *T. stocksianum* is affected by the different solvent types. Therefore, the current study investigates how different solvent systems of varying polarities influence the extraction efficiency, phytochemical content, and bioactivities of *T. stocksianum* extracts (leaves, roots, and stem). It was found that water and water-based organic solvent combinations gave the highest extraction yields; however, phytochemical contents and bioactivities were not directly proportional to extraction yields. The phenolic and flavonoid contents positively correlated with most solvents' miscibility indices except for water. Moreover, a blend of non-polar and polar solvents extracted higher amounts of flavonoid and phenolic contents than non-polar solvents alone. A direct correlation (r^2) was seen between phenolic contents and antioxidant activities across three plant parts. The best IC_{50} value was recorded for methanolic leaf extract (27 µg/mL), methanol-chloroform stem extract (37 µg/mL), and methanol-acetone root extract (34 µg/mL). Protein kinase enzyme inhibitory activity was highest in n-hexane-ethyl acetate leaf extract (15.7 ± 2.3 mm inhibition against *Streptomyces*), while the brine shrimp bioassay revealed potential cytotoxicity with LC_{50} values of 5 µg/mL (chloroform root extract), 16 µg/mL (acetone stem extract), and 47 µg/mL (n-hexane leaf extract). Extracts prepared using medium-polarity solvents (methanol, ethyl acetate, ethanol, and acetone) demonstrated excellent antifungal and antibacterial properties. In conclusion, the *T. stocksianum* extract's antioxidant activities and reducing power directly correlate with the phenolic contents and overall biological and cytotoxic activities.

Key words: *Teucrium stocksianum* Boiss; Solvent polarity; Phytochemical analysis; Antioxidant activities; Cytotoxicity; Protein kinase inhibition; Antimicrobial activities

Introduction

Teucrium stocksianum Boiss, a medicinally important specie in the family Lamiaceae, is a woody subshrub commonly found in hilly areas (Dir, Hazara, Malakand, and Swat) of Pakistan (Ahmad *et al.*, 2002). It is a densely branched herb with grey-green leaves with its height reaching up to 15-30 cm. In traditional medicine, its young branches and leaves are commonly prepared as teas or poultices to access their therapeutic benefits (Shah *et al.*, 2012). This medicinal herb has a long history as a folk remedy for treating multiple health conditions, including skin rashes, diabetes, burning feet syndrome (Ibrar &Hussain, 2009), cancer (Ullah *et al.*, 2018), hypertension, throat irritation and epilepsy (Ahmad *et al.*, 2002; Iqbal and Hamayun, 2004). Also, research studies have confirmed its crude leaf extracts for other biological activities, including antimicrobial, antispasmodic, and protective effects on the gastric system and liver (Rasheed *et al.*, 1995; Wasfi *et al.*, 1995; Islam *et al.*, 2002; Ali *et al.*, 2011; Rahim *et al.*, 2023).

The antioxidant defense mechanism is a protective barrier to the excess free radicals produced in the biological systems (Rosa *et al.*, 2007; Bhattacharya, 2015; Ifeanyi, 2018). Without antioxidant compounds, the excess free radicals produce oxidative stress that damages the living system (Sen *et al.*, 2010; Rao *et al.*, 2011; Sharma, 2014). They can attack nucleic acids, proteins, and lipids of cell membranes. As a result, they can easily cause the peroxidation of cell membranes that may lead to kidney diseases (Gwozdzinski *et al.*, 2021), cancer (Li *et al.*, 2013), diabetes (Memısoğullari *et al.*, 2003), arthritis, atherosclerosis (Khosravi *et al.*, 2019), reperfusion damage, and inflammation-like diseases (Biswas *et al.*, 2017; Maddu, 2019).

Plants synthesize structurally and chemically diverse bioactive compounds that have been found to have cytotoxic and antitumor activities (Raina *et al.*, 2014; Chudzik *et al.*, 2015; Al-Assar *et al.*, 2021). The major phytochemical classes synthesized by plants, such as phenolics and flavonoids, exert multiple biological effects (Ayoola *et al.*, 2008; Sahreen *et al.*, 2015), including anti-carcinogenic, anti-inflammatory, anti-thrombotic, hepato-protective, antimicrobial, anti-viral,

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anti-allergic and antioxidants to reduce cellular oxidative damage (Omar, 2010; Patel *et al.*, 2010; Gul *et al.*, 2011; Issazadeh *et al.*, 2012; Mehwish *et al.*, 2019). Given their protective effects, plant antioxidants are increasingly being studied for their potential in preventing oxidative damage and combating diseases like cancer (Prior *et al.*, 2005; Perveen & Al-Taweel, 2017). However, one bioassay is usually insufficient to comprehensively assess the antioxidant capacity of phyto-extracts, as it involves complex mechanisms. Moreover, the complex and labor-intensive nature of bioactivity assays, from preparing samples to analyzing data, presents a significant bottleneck in natural product research (Tariq *et. al.*, 2024). One powerful way to enhance the accuracy and efficiency of these tests is to refine the extraction process itself (Nida *et al.*, 2024). By employing a variety of organic solvents across the polarity spectrum, we can capture a much fuller range of a plant's bioactive compounds. This systematic approach does not just increase the diversity of isolated compounds; it helps identify the single most effective solvent for a specific plant, ultimately creating more dependable and efficient evaluation methods (Huang *et al.*, 2007; Nawaz *et al.*, 2019; Wakeel *et al.*, 2019). With this in mind, our study was designed to comprehensively analyze extracts from *T. stocksianum* three parts (roots, leaves, stem). We used fourteen different solvent systems to measure phenolics and flavonoids and to assess antioxidant and free radical quenching capabilities. The investigation was extended to evaluate cytotoxicity through a brine shrimp and protein kinase inhibition bioassays. While the antioxidant capacity of *T. stocksianum* has been noted before (Rahim *et al.*, 2013), a critical gap has remained: no research has explored how the choice of solvent impacts its broader therapeutic potential. Our work is the first to connect solvent polarity to the plant's antioxidant, antimicrobial, and cytotoxic outcomes. We, therefore, comprehensively studied the influence of 14 distinct polarity solvents on the antimicrobial, antioxidant, and cytotoxic potentiality of this medicinal shrub *In vitro*. To our knowledge, this is the first study aimed to thoroughly examine the leaves, stems, and root extracts of *T. stocksianum* using fourteen solvents of varying polarities along with their evaluation of a range of biological activities across different biological systems.

Materials and Methods

Collection and identification of plant: *Teucrium stocksianum* Boiss (roots, leaves, and stem) were sampled in separate bags in April-May, 2022 from the hilly region of Balambat, City Dir (Lower), Pakistan, and shifted to Nanobiotechnology Laboratory, Hazara University-Mansehra, Pakistan. The plant taxonomist identified and authenticated the plant as *Teucrium stocksianum* Boiss and was preserved as Voucher specimen NBHU-011.

Drying and extraction: The surfaces of the plant materials (roots, leaves, and stems) were cleaned by washing with deionized water, then shade-air-dried, and pulverized in a stone grinder. Cold maceration method was used for extraction. The extraction process began by mixing dried powder with 14 distinct solvent systems (Table 1) in 1:4, and the mixtures were allowed to stay for 24 hours. The mixtures were then vacuum filtered to obtain the solvent dissolved plant metabolites. The wet powder residue was re-suspended in fresh solvent for the same time period and filtered it once more. The liquid filtrates from both extractions were pooled together. The solvent was then carefully evaporated at 45°C, leaving the concentrated crude extract. Final weight of the crude extract was recorded to determine the extraction efficiency as a percentage, using the formula:

$$\% \text{ Extraction efficiency} = \left[\frac{\text{Weight of extract (g)}}{\text{Weight of powder used (g)}} \right] \times 100$$

Estimation of phenolics and flavonoids

Phenolic contents: The Folin-Ciocalteau reagent-based phenolics quantification was carried out for all 42 crude extracts (Yuan *et al.*, 2005). Samples to be tested (4 mg/mL) were combined with Folin-Ciocalteau phenol reagent (10 times diluted) and Na_2CO_3 (6%) in a 96-well microplate (25°C for 90 minutes). The absorbance was taken on a Tecan Plate Reader (Biotek ELX 800) at 630 nm. Methanol dissolved gallic acid (1 mg/mL) served as the calibration standard. Gallic Acid Equivalent (GAE) in µg per milligram (mg) of extract was the unit used for the phenolic contents estimation.

Table 1. Extraction efficiency of different polarity solvent in three parts of *Teucrium stocksianum*.

Solvent extraction systems	Polarity index	Extraction efficiency (%)		
		Leaves	Stem	Root
n-hexane	0.1	1.96	0.84	0.571
Chloroform	4.1	5.4	1.12	1.371
Ethyl acetate	4.4	4.36	2.12	1.4
Acetone	5.1	13.65	6.42	10.46
Ethanol	5.1	9.32	4.06	6.257
Methanol	5.2	*17.02	9.6	9.914
Water	9	*20.02	*13.06	8.429
n-hexane-ethyl acetate	4.4	4.4	1.44	1.371
Ethanol-n-hexane	5.2	6.92	2.6	2.914
Methanol-chloroform	9.2	13.76	6.84	8.771
Methanol-ethyl acetate	9.5	13.34	8.16	8.057
Methanol-acetone	10.2	13.58	6.92	10.09
Acetone-water	14.1	*18.76	*14.7	12.66
Methanol-water	14.1	*19.5	*14.82	*11.54

*Indicates the highest quantity of extract obtained in respective solvent from leaves, stem, and root parts

Flavonoid contents: Flavonoids were measured in all 42 crude extracts by adapting the protocol from (Basit *et al*., 2025). The samples (4 mg/mL) to be tested were combined with 1 M potassium acetate, 10% aluminium chloride aqueous solutions, and double distilled water (ddH_2O). After incubating (30 minutes, 37°C) the optical density was taken on a Tecan Plate Reader (BioTek ELX 800) at 415 nm. Methanol dissolved quercetin (1 mg/mL) served as the calibration standard. Equivalents of Quercetin (QE) in µg per mg of extract was the unit used for the flavonoid contents estimation.

Antioxidant activities: The antioxidant potential of *T. stocksianum* root, stem, and leaf solvent extracts (a total of 42 crude extracts) was estimated by performing three different assays.

DPPH assay: A DPPH assay optimized protocol from (Braca *et al.*, 2002) was followed to measure the radical neutralizing potential of all 42 crude extracts. Extract samples (20 µL) at four concentrations (200, 100, 50, and 20 µg/mL) were dispensed into the wells of 96-well plates, to which 180 µL of DPPH solution (OD = 1.00, at 515 nm) was introduced, and then incubated (1 hour, 25°C). Initially, the reaction was carried out with 200 µg/mL of sample, and then the concentration was reduced to determine the IC_{50} value. Dimethyl sulfoxide (DMSO) and ascorbic methanolic solution served as controls (negative and positive), and readings were monitored at wavelength of 515 nm. Percent scavenging was computed using the formula:

$$\% \text{ Scavenging} = \left[\frac{(\text{Control O.D} - \text{Sample O.D})}{(\text{Control O.D})} \right] \times 100$$

Reducing power (RP) evaluation via potassium ferricyanide reduction method: A previously optimized protocol (Wakeel *et al.*, 2019) was used to estimate reducing power, with ascorbic acid as a reference standard. 40 µL test samples (4 mg/mL) were combined with 50 µL Tripotassium hexacyanoferrate(III) (1%) and 50 µL of 200 mM sodium phosphate buffer (P^H 6.6), which were then incubated (50°C, 20 minutes), and then 50 µL of Trichloroethanoic acid (10%) was combined with it. The mixtures were then spun (3000 rpm, 10 minutes), and supernatant (166.66 µL) was separated in the wells of a 96-well plate, to which 0.1% ferric chloride (33.3 µL) was added. This plate was then incubated (2 hours, 25°C), and readings were monitored at 695 nm. The reduction of ferricyanide by each crude extract sample was quantified and reported as equivalents of µg L-Ascorbic Acid (AE) per mg of extract.

Total antioxidant capacity (TAC) measurement by phosphomolybdenum method: A protocol from (Prieto *et al.*, 1999) was slightly modified and adopted to estimate the TAC of 42 crude extracts. Extract samples (4 mg/mL) and ascorbic acid standard, each in 20 µL, were placed in separate tubes. Subsequently, 180 µL of the pre-prepared reagent (4 mM $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 600 mM H_2SO_4, 28mM Na_3PO_4) was dispensed to each tube. Following incubation (90 minutes, 95°C), the tubes were cooled down and the reaction mixtures were carefully transferred to microplate (96-well plate). 630 nm was the wavelength for monitoring absorbance. The TAC was presented as equivalents of µg L-Ascorbic Acid (AE) per mg of extract.

Cell toxicity bioassays

Brine shrimp bioassay: The cytotoxic nature of 42 crude extract samples was investigated quantitatively *in-vitro* following a previous protocol with slight modifications (Olowa & Nuñeza, 2013). First, seawater was prepared by mixing ddH_2O with sea salt (36 g/L) and dried yeast (6 mg/L) in a glass tank illuminated with an incandescent light source. Next, 1 g of *Artemia salina* eggs were introduced to the brine water-filled tank and incubated at 32ºC for 48 hours. Upon maturation and attraction to the light source, hatched nauplii were picked and dispended in a seawater beaker. 96-well plates were utilized to conduct this assay. Initially, the assay was performed with a 1000 µg/mL test sample, and then three other concentrations (100, 10, and 1 µg/mL) were used to estimate the LC_{50} value better. First, the calculated extract sample volume for each concentration was taken into each well. Then seawater (100 µL) was added to reduce the direct toxic effects of the extract on nauplii. Next, 10 hatched nauplii were introduced to each well, and the volume was brought up to 300 µL to reach the desired concentration. The plate was incubated for 24 hours, and following this, the live and dead shrimps were counted. The mortality percentage was computed by dividing the dead nauplii count in each well by the total count and multiplying by 100.

Cell-based protein kinase bioassay: This assay utilizes a *Streptomyces* (strain 85E), which sporulates readily when grown on specific agar media. 5 µL of samples (20 mg/mL) to be tested were applied to filter paper discs and placed onto ISP4 agar plates inoculated with freshly spread *Streptomyces* 85E spores as explained by (Waters *et al.*, 2002). After the incubation at 28°C for 3−7 days, aerial mycelium formation and sporulation were carefully observed. Those extract samples inhibiting the sporulation but not mycelium growth were considered active. This technique provides an inexpensive, cell-based method for screening bioactive extracts, with the benefit of differentiating compounds specifically targeting sporulation.

Antimicrobial assays

Antifungal assay: The antifungal effects of the 42 crude extracts were carried out via a well-known agar disc diffusion method (Sharma *et al.*, 2009). First, pathogenic strains of fungi (*Mucor* sp. FCBP-0300, *Aspergillus flavus* FCBP-0064, *Aspergillus fumigatus* FCBP- 66) spores were suspended in a 0.02% Polysorbate 20 solution to adjust the turbidity to a 0.5 McFarland (McF) reference. Next, this spore suspension was spread onto agar plates (Sabouraud Dextrose Agar), and pre-prepared Whatman discs, with 5 µL of absorbed sample extract (20 mg/mL), were placed on them. Clotrimazole and DMSO-loaded Whatman discs were taken as controls (positive and negative). Following incubation at 28°C for 28-48 hours, the inhibitory zones were first confirmed via visual observation and then measured with the help Vernier Calliper in unit of millimetre (mm).

Minimum inhibitory concentration (MIC): A 45 µL volume of extract samples at four concentrations (100, 33.3, 11.1, and 3.7 µg/mL) in nutrient broth were dispensed to the wells of a microplate, each pre-filled with 5 µL of DMSO. One well was filled only with 50 µL of nutrient broth plus DMSO, serving as the negative control. Subsequently, fungal spore inoculum (50 µL) at 0.5 McF was introduced to the wells, bringing the total volume to 100 µL/well. The plate was then placed inside the incubator (28°C, 48 hours), after which it was agitated. Absorbance was then monitored on an ELx800 automated plate reader (Botek) at 450 nm. Clotrimazole was the positive control. The least concentration of extract that caused a reduction or complete cessation of fungal growth was its MIC. Percent activity was calculated using the formula adopted from (de Felício *et al.*, 2010):

$$\% \, Inhibition = \left[1 - \frac{AE - AEB}{AC - ACB}\right] \times 100$$

AEB refers to the optical density (OD) of plates with medium and the sample, AE represents the optical density (OD) of the tested plates, AC signifies the OD of plates with the negative control, and ACB denotes the OD of plates with only nutrient broth.

Antibacterial assay: To determine the antibacterial effects of extract samples, the agar disc diffusion method was utilized, and four different bacterial pathogens were used in the study (*Salmonella typhimurium* ATCC-14028, *Pseudomonas aeruginosa* ATCC-27853, *Klebsiella pneumoniae* ATCC-1705, *Escherichia coli* ATCC-25922). These pathogens were cultured overnight, and their turbidity was adjusted to 0.5 McF, followed by the spreading of this inoculum on the surface of nutrient agar plates. Next, Whatman paper discs, with absorbed 5 µL of test samples (20 mg/mL), were set horizontally on the media surface and then plates were positioned inside the incubator (37 °C for 24 hours). DMSO and cefixime were controls (negative and positive). Vernier caliper was the instrument used to record the zones in mm. Those extracts representing an inhibition zone of ≥ 10 mm were considered active and proceeded for MIC determination.

Minimum inhibitory concentration (MIC): A previously optimized high-throughput 96-well microplate method from (Sultanbawa *et al.*, 2009) was utilized for MIC determination. Extract samples in nutrient broth were sequentially diluted to reach concentrations of 100, 33.3, 11.1, and 3.7 µg/mL, followed by loading into a sterile 96-well plate. Next, bacterial inoculum was dispensed to the wells to a final density of 5×10^4 CFU/mL. Two columns of wells were filled only with inoculum and nutrient broth, serving as a negative control. Cefixime monohydrate served as a positive control. Once the samples were loaded, the initial absorbance (t0) was measured at 630 nm. After this, plates were incubated (37°C, 22 hours), then gently agitated, and the final optical density (t22) was taken at a specific wavelength (630 nm). The least concentration of extract that caused a reduction or complete cessation of bacterial growth was its MIC. Inhibition percentage was computed using the given equation (Sultanbawa *et al.*, 2009):

$$\% \text{ inhibition} = [1 - \frac{(\text{t22} - \text{t0})}{(\text{C22} - \text{C0})}] \times 100$$

C0 are the OD values for the negative control at t0, while C22 are the OD values of the negative control at t22.

Data analysis: The assays were executed in triplicate, and the obtained data was analyzed using the software (Microsoft Excel 2013). IC_{50} values were determined with Table Curve Software, and the results are displayed as Average ± SD in both tables and graphs.

Results and Discussion

Influence of solvent's polarity on extraction efficiency: The extraction procedure and solvent extraction system selection play a key part in isolating important secondary metabolites (phenolics and flavonoids) and their antioxidant activities (Lezoul *et al.*, 2020; Monteiro *et al.*, 2020). Therefore, using the distinct solvent systems with different polarities extracts the different levels of these secondary metabolites. In the current study, fourteen different polarity solvent systems were used to extract secondary metabolites of *T. stocksianum*, and their percent yield is presented in Table 1. Our findings indicate that the extraction efficiency increases with the polarity of the solvent. However, when different polarity solvents were used in the 1:1 ratio combination, they either inhibited each other percent yield or increased it. In our study, we achieved a higher yield in all three parts (leaves, stem, roots) of the plant in water and a 1:1 combination system of acetone-water and methanol-water. The table shows that a combination of acetone-water, methanol-water, and water alone produces more yield than other solvents. This demonstrates a positive correlation between the extraction yield and the solvent's polarity. However, in some cases, the polar and nonpolar solvents antagonistically reduced the extraction efficiency (ethanol-n-hexane; n-hexane-ethyl acetate), or they did not increase the extraction yield when polar and nonpolar solvents were used in a 1:1 ratio combination, as shown in Table 1. However, factors such as the powder-to-solvent ratio, particle size, solvent's kind, and contact time all influence the extraction yield (Chirinos *et al.*, 2007; Addai *et al.*, 2013; Mohamad *et al.*, 2013). In current study, the powder-to-solvent ratio, contact time, and solvent's kind were kept constant, with the only variation being the particle size. The leaf's particle size was finer than the stem and stem from the root. Therefore, in most cases, the leaves gave the highest extraction yield.

Estimation of phenolics and flavonoids

Phenolic contents: The phenolics extracted by fourteen different solvents from three different parts of the plant are plotted in Fig. 1A. From the graph, it is clear that phenolic contents vary from polar to non-polar solvents because different solvents extract different kinds and concentrations of phenolic compounds. Polar solvents have high solubility indices and, therefore, extract a high amount of phenolic compounds (Canadanovic-Brunet *et al.*, 2008; Mohsen & Ammar, 2009; Wakeel *et al.*, 2019). We observed the same trend in which the polarity index was positively related to the quantity extracted phenolic compounds. Interestingly, when the polarity index

increased from 5.2 (ethanol) to 9 (water), the phenolic content decreased instead of increasing. In all three plant parts, water and water-combination solvent extracts, which have high polarity indices, showed fewer polyphenols, indicating that water only extracts water-soluble polar compounds (Mohammedi and Atik, 2011; Dent *et al.*, 2013). The maximum total phenolic contents for leaves and stem plant material were observed in acetone and methanol fractions. In contrast, the highest phenolics for roots were seen in the ethanol and acetone fractions. When the solvents were combined in the 1:1, the methanol-acetone combination and methanol-chloroform combination showed the highest quantity of phenolics in all three plant parts.

Flavonoid contents: Flavonoids constitute a diverse class of secondary metabolites in plants, distinguished by their phenolic hydroxyl group, and are found throughout various plant parts. Flavonoids are considered strong antioxidants that effectively scavenge oxygen-reactive species (Anokwuru *et al.*, 2011; Treml & Šmejkal, 2016; Hernández-Rodríguez *et al.*, 2019; Kaurinovic & Vastag, 2019). The total flavonoid contents in each crude extract of three different plant parts are presented in Fig. 1B. The findings also show that total flavonoid content generally increases with solvent polarity. However, in the case of water and its combinations with other solvents, the exceptionally high polarity does not lead to a higher flavonoid content. Water gave a high extraction yield, but extracts only a limited kinds of phyto-compounds in leaf and stem extracts. When the combination of polar and nonpolar solvents was used, again, the highest polarity solvent extracted more flavonoids than the nonpolar solvent. However, no significant variations were noticed in the flavonoid content of the roots in the lower polar solvents, although a slight variation was observed in the combination of solvents. Overall, in all three parts, ethanol, methanol-acetone, acetone, methanol, and methanol-ethyl acetate represented the highest content of flavonoids.

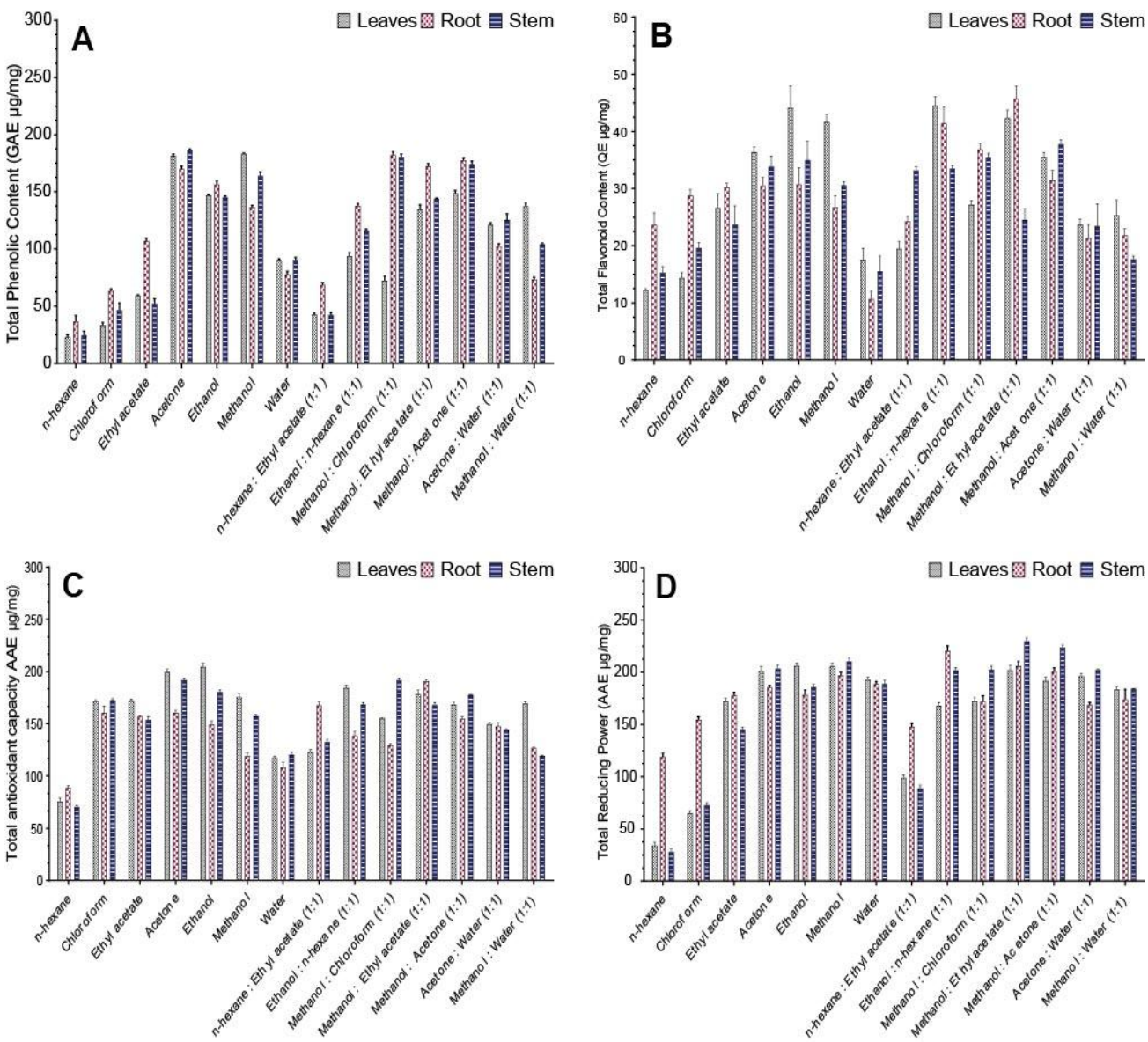

Fig. 1. Effect of 14 different solvent systems on Phytochemicals content, antioxidant and reducing power activities. **(A)** Total phenolic content **(B)** Total flavonoid content **(C)** Total antioxidant capacity **(D)** Total reducing power in roots, stems, and leaves extracts of *Teucrium stocksianum*.

Table 2. IC_{50} value of DPPH radical scavenging activities in *T. stocksianum*.

Solvents systems	Leaves		Stem		Roots	
	DPPH scavenging at 200 µg/mL	IC_{50} µg/mL	DPPH scavenging at 200 µg/mL	IC_{50} µg/mL	DPPH scavenging at 200 µg/mL	IC_{50} µg/mL
n-hexane	13 %	>500	18 %	>500	30 %	349
Chloroform	11 %	>200	19 %	>500	60 %	168
Ethyl acetate	42 %	301	35 %	>500	92 %	75
Acetone	92 %	*42	92 %	*42	91 %	*41
Ethanol	92 %	55	93 %	*44	92 %	*39
Methanol	92 %	*27	92 %	54	92 %	*41
Water	83 %	87	92 %	107	83 %	102
n-hexane-ethyl acetate	29 %	>500	26 %	480	60 %	164
Ethanol-n-hexane	78 %	102	92 %	66	92 %	44
Methanol-chloroform	71 %	117	92 %	*37	91 %	49
Methanol-ethyl acetate	92 %	*34	93 %	58	92 %	46
Methanol-acetone	92 %	*43	93 %	41	93 %	*34
Acetone-water	92 %	57	90 %	46	90 %	75
Methanol-water	92 %	56	91 %	64	82 %	131

*Indicate the IC_{50} (µg/mL) value showing best DDPH scavenging activities in the extract obtained in respective solvent from leaves, stem, and roots

Antioxidant potential tests

DPPH assay: The neutralization capacity of each crude extract was calculated as IC_{50}, representing the concentration in µg/mL that is needed to neutralizes DPPH radical to non-radical form by 50%. IC_{50} values were computed using TableCurve program and Tabulated in Table 2. Overall, each plant part showed good scavenging effects. However, the scavenging activities in different solvent extracts represented varying levels of inhibition. The best IC_{50} value was estimated at 27, 34, and 37 µg/mL in leaves methanolic extract, root methanol-acetone extract, and stem methanol-chloroform extract, respectively. Comparatively, the n-hexane, chloroform, and ethyl acetate have displayed poor IC_{50} values in stem extracts. The combination of n-hexane-ethyl acetate also did not represent good scavenging activity in the stem and leaves. Generally, the extracts from all plant parts prepared with different solvent systems exhibited the most potent antioxidant activity, as indicated by IC_{50} values typically below 150 µg/mL. We also observed that the percent scavenging activities increases as we increases the concentration of the extract. This is in close agreement with a previous studying indicating increase activities with increase in concentration (Zaib *et al*., 2024). When comparing the different plant parts, the roots clearly emerged as the most potent source of antioxidants, significantly outperforming both the stems and leaves.

Total antioxidant capacity (TAC): TAC is a crucial metric for evaluating the antioxidant strength of phyto-extracts (Ghoora *et al.*, 2020). In this study, *T. stocksianum* TAC values for the leaves, stems, and roots consistently mirrored the trends seen in the total reducing power assay (Fig. 1C). When examined the solvent extracts, distinct patterns emerged. For polar solvents, ethanol yielded the highest TAC values, despite being less polar than water or methanol. For non-polar solvents, the antioxidant capacity increased directly with the solvent's polarity, making acetone the most effective. The mixed solvent systems, however, showed no clear relationship with polarity; instead, a methanol-ethyl acetate blend consistently produced the highest TAC for every plant part. Ultimately, regardless of the solvent system, the leaves always generated the highest TAC values, followed by the stems and roots, confirming that the leaves are the richest source of antioxidants in this plant.

Reducing power assay: The ferric ion reduction assay effectively demonstrated the significant antioxidant effect of the plant extracts. As is well-established, an extract's reducing power is directly linked to its antioxidant activity (Jayanthi & Lalitha, 2011), and our findings were no exception. The results revealed a consistent and predictable trend across the leaves, stems, and roots (Fig. 1D). Specifically, the reducing power grew stronger with increasing solvent polarity; this trend held true for most solvents but notably deviated when water was used, either alone or in a mixture. This reinforces that reducing capacity is a robust measure of antioxidant potential in *T. stocksianum*. The data clearly identifies that methanol, ethanol, and acetone were superior at extracting these potent reducing agents.

Correlative relationship between phenolic and flavonoid contents: The results of our correlation analysis clearly establish phenolic content as the primary driver of antioxidant activity in *T. stocksianum*. Across all fourteen solvent extracts from the leaves, stems, and roots, we observed a strong link: extracts rich in phenolics consistently showed superior TAC and stronger reducing power (Table 3, Fig. 2A-C). The study also examined correlations within the fourteen solvent extracts and between the three plant parts. In leaf extracts, phenolic content strongly correlated with total antioxidant capacity (r^2=0.646699) and reducing power (r^2=0.828064). Similarly, stem extracts exhibited correlation values r^2 = 0.697138 and r^2 = 0.864011, respectively. In contrast, root extracts demonstrated weaker correlation values (r^2= 0.425534 and r^2=0.695479). Across all parts, reducing power consistently positively correlated with phenolic content, supporting the idea that high phenolic contents are responsible for enhanced antioxidant potential. These findings not only aligns with previous research confirming phenolics as key antioxidants (Wang *et al.*, 2010); (Zhang *et al.*, 2010), but also explains the powerful reducing capabilities we observed in leafs and stem (Rodríguez Madrera *et al.*, 2021). Furthermore, these findings highlights precisely why solvent choice is so critical (Fig. 2). The solvent polarity dictates the efficiency of phenolic extraction and, in turn, the final antioxidant strength of the extract (El-Chaghaby *et al.*, 2014; Kaczorová *et al.*, 2021). As a final point of interest, the two antioxidant metrics themselves, TAC and reducing power, were moderately correlated in the leaves (r^2 = 0.63) and stems (r^2 = 0.58), but poorly correlated in the roots (r^2 = 0.32).

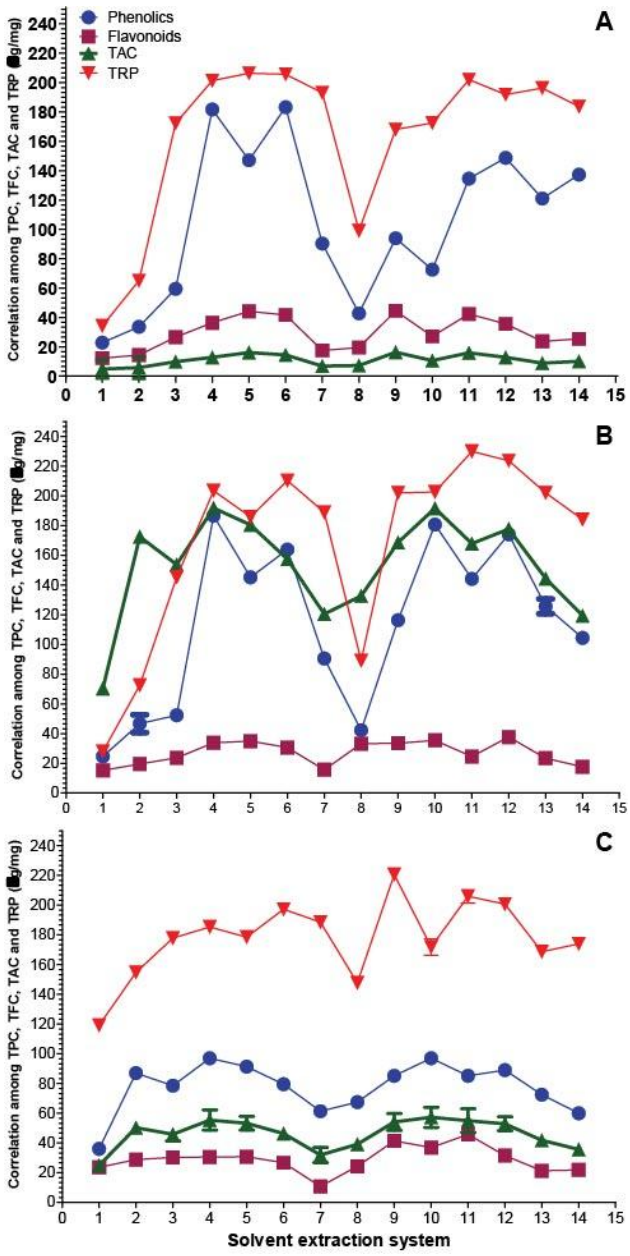

Fig. 2. Effect of Total phenolic and flavonoid contents on its antioxidant and reducing power activities in (A) Leaves (B) Stem (C) Roots of *Teucrium stocksianum* Boiss in 14 different Solvents extraction system. Solvent system on X-Axis are; 1. n-hexane 2. Chloroform 3. Ethyl acetate 4. Acetone 5. Ethanol 6. Methanol 7. Water 8.n-hexane-Ethyl acetate 9. Ethanol -n-hexane 10. Methanol-Chloroform 11. Methanol-Ethyl acetate 12. Methanol –Acetone 13. Acetone-Water 14. Methanol-Water.

Cytotoxicity assays

Protein kinase inhibition assay: *Streptomyces* is a key microbe used to screen protein kinase inhibitors, which are compounds that target protein kinases and suppress the cancer cells growth and spread. These compounds also inhibit the growth of *Streptomyces griseus* aerial hyphae by disrupting cellular differentiation and protein phosphorylation while leaving substrate mycelial growth unchanged (Hong *et al.*, 1993). This suggests that *S. griseus* carries eukaryotic-like protein kinases play a key function in their secondary metabolism and morphogenesis. Based on this, a screening method was proposed that utilizes prokaryotic indicator strain to discover protein kinase inhibitors that could be used for treating cancer (Hong *et al.*, 1993; Wrigley *et al.*, 2000).

The present study used *Streptomyces* as test organisms against fourteen different solvent extracts to find compounds that could be used as potential protein kinase inhibitors. Two types of zones appeared on the culture plate: the bald zone and the clear zone. The bald zone indicates that the fraction/extract inhibits protein kinase produced by the test strain, whereas the clear zone indicates cytotoxicity. We found that some of the extracts inhibited the production of protein kinase (bald zone), and some fractions showed cytotoxic activities (clear zone). Among the three parts, leaves demonstrated inhibitory activity on the *Streptomyces* growth, whereas shoot and root extracts were mostly cytotoxic (Table 4). Moreover, no significant variances were detected in the activities of polar and nonpolar solvent extracts. The maximum activity of 15.7±2.3 mm was calculated for n-hexane-ethyl acetate extract in leaves, followed by 12.8±1.24 mm in water extract of stem, and 13±1.7 mm in ethyl acetate extract of roots.

Brine shrimp bioassay: This assay indicates that the plant solvent extracts effectively killed the nauplii in the growing state. The LC_{50} value of each extract in each plant part was also calculated using the Table curve software. The best LC_{50} value was calculated 47 µg/mL in the n-hexane extract of leaves, 16 µg/mL in the acetone stem extract, and 8, 7, 15, 12, 13, and 5 µg/mL in the methanol-chloroform, chloroform, n-hexane-ethyl acetate, ethanolic, ethanol-n-hexane, and methanol-ethyl acetate extracts of roots respectively (Table 5). Overall, root extracts showed lowest LC_{50} values than leaves and stem extracts, indicating that a small amount of root extract is enough for lethality. The slightly higher LC_{50} values of leaves and stem extracts indicate that these extracts are not toxic to cells in much higher quantities.

Antifungal activities of *T. stocksianum* extracts: The antifungal efficacy of *T. stocksianum* solvent extracts was evaluated against four distinct fungal pathogens. The solvent extracts fungal growth inhibitory effects are listed in Table 6. According to data, *Mucor* spp. was found to be the most resistant strain, whereas *A. niger* was observed most susceptible strain to the solvent extracts of leaves, roots, and stem. The highest zone (15±1.32 mm) against *A. niger* was recorded in the leaves' chloroform extract, 14±1.5 mm in the stem's ethyl acetate extract, 19±4.2 mm, and 18±2.1 mm in the n-hexane-ethyl acetate and ethyl acetate extract of roots, respectively. Similarly, 12±2.6 mm and 16±3.4 mm zones were recorded against *A. fumigatus* in the leaves and roots' n-hexane-ethyl acetate extract and 15±2.12 mm in the roots' methanol-acetone extract. In contrast, none of the stem extracts represented antifungal activity against *A. fumigatus.* Regarding leaf solvent extracts, no antifungal activity was observed against *A. flavus*, whereas 10±2.1 mm and 10±1.5 mm inhibition zones were recorded for the acetone-water extracts and stem's n-hexane-ethyl acetate, respectively. Across all extracts, ethanol-n-hexane root extract showed the highest activities (20±0.98 mm). Based on the antifungal activities, we conclude that not all solvents are equally effective against the selected strains in all plant parts. Therefore, optimizing the protocol for each fungal strain with appropriate solvents is recommended. The root extracts showed the strongest antifungal activity among all parts, which is attributed to its distinct composition of phytocompounds, including flavonoids and phenolics and their concentration (Lattanzio *et al.*, 2009; Swallah *et al.*, 2020). Therefore, researchers should investigate the impact of both solvent types and medicinal plants different parts on secondary metabolite extraction to identify lead compounds.

Table 3. Correlation (r^2) between phenolic content and its antioxidant activities in leaves, stem, and roots extracts of *Teucrium stocksianum.*

	TPC	TFC	TAC	TRP
	Correlation (R^2) among leaves extracts			
TPC	-	-	-	-
TFC	0.74389	-	-	-
TAC	0.646699	0.768723	-	-
TRP	0.828064	0.710043	0.634109	-
	Correlation (R^2) among stem extracts			
TPC	-	-	-	-
TFC	0.641808	-	-	-
TAC	0.697138	0.735464	-	-
TRP	0.864011	0.453454	0.589729	-
	Correlation (R^2) among roots extracts			
TPC	-	-	-	-
TFC	0.665418	-	-	-
TAC	0.425534	0.549452	-	-
TRP	0.695479	0.452812	0.321098	-

TPC (total phenolic contents), TFC (total flavonoid contents), TAC (Total antioxidant capacity), TRP (total reducing power)

Table 4. Protein kinase inhibition assay using culture of *Streptomyces* 85E strain.

Solvent system	Protein kinase ınhibition at a concentration of 20 mg/mL stock solution of sample (mm ± SD)					
	Leaves		Stem		Root	
	Zone (mm)	Type of zone	Zone (mm)	Type of zone	Zone (mm)	Type of zone
n-hexane	7.2 ± 2.04^{b}	(Bald zone)	8.3 ± 2.8^{ab}	(Clear zone)	8.8 ±3.1^{ab}	(Clear zone)
Chloroform	6.3 ± 1.9 ^{b}	(Bald zone)	7.4 ± 1.29^{b}	(Clear zone)	7.6 ±3.3^{b}	(Clear zone)
Ethyl acetate	9.1 ± 1.2a^{b}	(Bald zone)	11 ± 1.42^{a}	(Clear zone)	13± 1.7 ^{a}	(Clear zone)
Acetone	8.6 ± 1.7 ^{ab}	(Clear zone)	9.2 ± 1.3^{ab}	(Clear zone)	9± 2.8^{ab}	(Clear zone)
Ethanol	12.3 ± 2.1^{a}	(Bald zone)	10.4 ± 1.5^{ab}	(Clear zone)	11± 1.4 ^{a}	(Bald zone)
Methanol	11 ± 1.7^{a}	(Clear zone)	11.4 ± 2.9^{a}	(Clear zone)	12± 1.2^{a}	(Clear zone)
Water	8.6 ± 1.3 ^{ab}	(Clear zone)	12.8 ± 1.24^{a}	(Clear zone)	10± 0.51^{ab}	(Clear zone)
n-hexane-ethyl acetate	15.7 ± 2.3 ^{b}	(Clear zone)	6.6 ± 2.3^{b}	(Clear zone)	9± 1.5^{ab}	(Clear zone)
Ethanol-n-hexane	6.4 ± 1.9^{b}	(Clear zone)	7.6 ± 2.5^{b}	(Bald zone)	7.9 ±1.3^{a}	(Clear zone)
Methanol-chloroform	7.6 ± 2.1^{b}	(Clear zone)	9.5 ± 3.6 ^{ab}	(Clear zone)	8± 2.31^{ab}	(Clear zone)
Methanol-ethyl acetate	10 ± 1.8^{ab}	(Clear zone)	9.8 ± 2.7 ^{ab}	(Clear zone)	9.0 ± 1.2^{ab}	(Bald zone)
Methanol-acetone	9.2 ± 2.5 ^{ab}	(Clear zone)	8.4 ± 1.3 ^{ab}	(Clear zone)	9.0 ± 1.6^{ab}	(Clear zone)
Acetone-water	7.4 ± 1.7 ^{b}	(Clear zone)	8.2 ± 1.6 ^{ab}	(Clear zone)	6.4 ± 1.41^{b}	(Clear zone)
Methanol-water	8.3 ± 1.6 ^{ab}	(Clear zone)	9.4 ± 2.8 ^{ab}	(Clear zone)	8.9 ± 1.8 ^{ab}	(Clear zone)
DMSO*	**2.34***		**3.78***		**2.11***	

*These values of negative control (DMSO) are subtracted from the original value in the table. Common letters in superscript indicate no significant difference in the activities, whereas single letters indicate significant differences in activities. mm (millimeter), SD (standard deviation). Clear zone indicates cytotoxicity, whereas bald zone indicates inhibitory effect

Table 5. Determination of lethal concentration (LC) that kill 50 % of brine shrimps by different solvent extracts of leaves, stem and root parts of *Teucrium stocksianum*.

Solvent	% Lethality at conc. 1000 µg/mL and LC_{50} (µg/mL)					
	Leaves		Stem		Roots	
	Conc. 1000 µg/mL	LC_{50} µg/mL	Conc. 1000 µg/mL	LC_{50} µg/mL	Conc. 1000 µg/mL	LC_{50} µg/mL
n-hexane	60 %	47	46 %	>500	45 %	>500
Chloroform	86 %	118	60 %	74	91 %	5
Ethyl acetate	88 %	132	77 %	102	88 %	213
Acetone	96 %	53	70 %	16	65 %	148
Ethanol	90 %	220	96 %	188	75 %	15
Methanol	58 %	>500	65 %	>500	59 %	129
Water	80 %	48	46 %	>500	45 %	>500
n-hexane-ethyl acetate	63 %	388	59 %	131	78 %	7
Ethanol -n-hexane	80 %	88	50 %	71	85 %	12
Methanol-chloroform	91 %	56	40 %	>500	88 %	8
Methanol-Ethyl acetate	54 %	>500	61 %	297	96 %	13
Methanol -Acetone	57 %	460	48 %	>500	90 %	158
Acetone-Water	55 %	>500	55 %	>500	55 %	450
Methanol-Water	55 %	49	57 %	222	40 %	>500

Table 6. Antifungal activities of the *Teucrium stocksianum* extracts against four fungal strains.

Solvent type	*Mucor* spp.		*Aspergillus niger*		*Aspergillus fumigatus*		*Aspergillus flavus*	
	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)
	***Teucrium stocksianum* leaves extracts**							
n-hexane	-	-	7 ± 2	-	6 ± 1.4	-	-	-
Chloroform		-	15 ± 1.32	33.33	7 ± 2	-	-	-
Ethyl acetate	-	-	12 ± 0.65	100	-	-	-	-
Acetone	-	-	-	-	-	-	-	-
Ethanol	9 ± 1.5	100	6 ± 1.3	-	-	-	-	-
Methanol	10 ± 2.35	100	8 ± 3.4	100	7 ± 3.05	-	-	-
Water	-	-	-		-	-	-	-
n-hexane-ethyl acetate	-	-	14 ± 0.96	100	12 ± 2.6	100	-	-
Ethanol -n-hexane	-	-	9 ± 2.4	100	7 ± 3.5	-	-	-
Methanol-chloroform	-	-	8 ± 2.1	-	10 ± 2	100	-	-
Methanol-ethyl acetate	-	-	7 ± 2.3	-	-	-	-	-
Methanol –acetone	-	-	-	-	8 ± 0.43	-	-	-
Acetone-water	-	-	-	-	-	-	-	-
Methanol-water	-	-	8 ± 1.3	-	8 ± 0.43	-	-	-
	***Teucrium stocksianum* stem extracts**							
n-hexane	-	-	10 ± 1.2	100	-	-	9 ± 1.32	100
Chloroform	-	-	13 ± 1.8	100	-	-	8 ± 1.3	-
Ethyl acetate	-	-	14 ± 1.5	33.33	-	-	11 ± 2.3	100
Acetone	-	-	-	-	-	-	-	-
Ethanol	-	-	10 ± 1.5	100	-	-	6 ± 1.6	-
Methanol	-	-	7 ± 1.4	-	-	-	7 ± 1.24	-
Water	-	-	-	-	-	-	6 ± 0.12	-
n-hexane-ethyl acetate	-	-	12 ± 3	100	-	-	10 ± 1.5	100
Ethanol -n-hexane	-	-	7 ± 0.6	-	-	-	7 ± 1.23	-
Methanol-chloroform	-	-	-	-	-	-	-	-
Methanol-ethyl acetate	-	-	-	-	-	-	6 ± 2.12	-
Methanol –acetone	-	-	10 ± 1.2	-	-	-	8 ± 1.43	-
Acetone-water	-	-	-	-	-	-	10 ± 2.16	100
Methanol-water	-	-	8 ± 2.3	-	-	-	7 ± 2.54	-
	***Teucrium stocksianum* root extracts**							
n-hexane	-	-	15 ± 2.32	33.33	10 ± 1.55	100	14 ± 0.9	33.33
Chloroform	-	-	12 ± 2.2	100	10 ± 2.7	100	18 ± 1.5	33.33
Ethyl acetate	-	-	18 ± 2.1	33.33	12 ± 0.45	100	-	-
Acetone	-	-	10 ± 2.9	100	-	-	11 ± 3.5	100
Ethanol	-	-	11 ± 2.5	100	8 ± 0.75	-	15 ± 2.12	33.33
Methanol	-	-	14 ± 1.4	33.33	7 ± 1.38	-	9 ± 1.9	100
Water	-	-	-	-	-	-	-	-
n-hexane-Ethyl acetate	-	-	19 ± 4.2	33.33	16 ± 3.4	33.33	-	-
Ethanol -n-hexane	-	-	12 ± 2.6	100	12 ± 1.6	100	20 ± 0.98	33.33
Methanol-Chloroform	-	-	11 ± 1.6	100	4 ± 0.21	-	12 ± 0.85	100
Methanol-Ethyl acetate	5 ± 0.5	-	9± 1 .98	100	11 ± 0.4	100	15 ± 2.5	33.33
Methanol –Acetone	-	-	13 ± 2.1	33.33	15 ± 2.12	33.33	7 ± 0.45	-
Acetone-Water	8 ± 1.7	-	10 ± 1.5	100	7 ± 2.1	-	3 ± 0.34	-
Methanol-Water	9 ± 1.1	100	10 ± 2.6	100	-	-	4 ± 0.75	-
Clotrimazol	20 ± 2.77		22 ± 0.32		21 ± 0.65		0 ± 0.11	-

The extract samples were used at 100 µg/mL (5µL). Values (mean ± SD) are the average of three samples of each extract. The sign (-) represents no activity at all

Antibacterial potential of *T. stocksianum* extracts: Screening different fractions from medicinal plant solvent extracts against pathogens is necessary to develop new drugs. This study extensively studied the antibacterial potential of fourteen different extracts of three parts (leaves, stems, and roots) of *T. stocksianum* against bacterial pathogens from both bacterial classes (gram-positive and negative). As detailed in Table 7, the extracts have substantial activities against both classes of bacterial pathogens. The maximum antibacterial activities in leaves extracts were 16±1.8 mm in methanolic extract against *P. aeruginosa*, 15±2.5 mm, 17±1.07 mm, and 16±2.6 in the acetone extract for *K. pneumoniae*, *E. coli*, and *S. typhi*, respectively, and 16±2.1 mm in the ethanolic extract against *E. coli*. Similarly, among the stem extracts, the ethyl acetate and ethanolic extracts represented better activities than other solvent extracts. We also observed that root extracts were mostly excellent in activities to all bacterial pathogens, especially *E. coli* and *S. typhi*, against which the highest activities were recorded, indicating the root extracts as the best source of active compounds against these two strains from *T. stocksianum*. Moreover, the extracts' activity significantly depends on the solvent system utilized to extract secondary metabolites, and moderate polar solvents represented excellent antibacterial activities. According to Fazal *et al*., (Fazal *et al.*, 2011) and Igbinosa *et al*., (Igbinosa *et al*., 2009), polar solvents allow the extraction of approximately all phenolic compounds, leading to good antibacterial activities. However, our study revealed good antibacterial activities in both polar (ethanol, Ethyl acetate, methanol) and non-polar (chloroform, acetone) solvent extracts, which are conceding to the previous results of Ahmad *et al*., (Ahmad-Khair *et al.*, 2024). This varying degree of susceptibility indicates that polar and non-polar solvents extract different types and concentrations of bioactive substances (Hayouni *et al*., 2007; Mukherjee *et al*., 2024). Therefore, optimizing extraction protocol is a prerequisite in any experimental procedure for bioactive compound extraction.

Table 7. Antibacterial activities of *Teucrium stocksianum* extracts against four bacterial strains.

Solvent type	*Pseudomonas aeruginosa*		*Klebsiella pneumoniae*		*Escherichia coli*		*Salmonella typhi*	
	*Zone (mm)	MIC (µg/mL)	*Zone (mm)	MIC (µg/mL)	*Zone (mm)	MIC (µg/mL)	*Zone (mm)	MIC (µg/mL)
	***Teucrium stocksianum* leaves extracts**							
n-hexane	6 ± 2.8	-	8± 2.4	100	13 ± 2.2	33.33	8 ± 2.22	100
Chloroform	13 ± 1.2	11.11	12± 3.2	100	12 ± 2.6	3.7	8 ± 1.3	-
Ethyl acetate	12 ± 0.5	33.33	14± 1.02	33.33	15 ± 3.4	11.11	11 ± 2.3	100
Acetone	14 ± 0.29	100	15± 2.5	100	17 ± 1.07	3.7	16 ± 2.6	3.7
Ethanol	13 ± 1.76	-	13± 1.6	11.11	16 ± 2.1	100	15 ± 2.8	11.11
Methanol	16 ± 1.8	33.33	12± 2.2	11.11	15 ± 1.33	-	12 ± 1. 4	100
Water	12 ± 1.6	100	10± 2.4	100	12 ± 2.1	100	10 ± 2.2	33.33
n-hexane-Ethyl acetate	14 ± 1.1	3.7	9± 2.6	33.33	10 ± 0.6	33.33	14 ± 1.5	33.33
Ethanol -n-hexane	9 ± 2.4	100	6± 1.25	-	8 ± 0.9	-	10 ± 1.23	33.33
Methanol-Chloroform	8 ± 2.1	-	11 ± 1.2	33.33	6 ± 2.4	-	12 ± 2.2	100
Methanol-Ethyl acetate	8 ± 1.04	-	6 ± 2.5	-	12 ± 1.1	100	10 ± 1.5	100
Methanol -Acetone	-	-	12 ± 0.43	100	9 ± 1.2	100	8 ± 1.3	-
Acetone-Water	11 ± 2	100	-	-	-	-	14 ± 0.56	3.7
Methanol-Water	10 ± 1.3	100	13 ± 0.43	33.33	9±2.8	100	6 ± 1.4	-
	***Teucrium stocksianum* stem extracts**							
n-hexane	10 ± 0.8	-100	6 ± 1.9	-	10 ± 0.52	100	8 ± 1.02	-
Chloroform	14 ± 0.6	33.33	10 ± 1.6	100	12 ± 0.16	11.11	10 ± 0.92	100
Ethyl acetate	16 ± 1.2	3.7	13 ± 1.22	33.33	16 ± 0.14	100	13 ± 0.13	100
Acetone	11 ± 2	100	12.6 ± 1.39	11.11	15 ± 0.37	11.11	12 ± 0.76	11.1
Ethanol	13 ± 0.86	33.33	12 ± 1.4	11.11	12 ± 0.11	11.11	10 ± 1.28	100
Methanol	14 ± 1.32	33.33	15 ± 0.32	3.7	14 ± 2.45	-	12 ± 0.94	33.33
Water	10 ± 0.46	100	11 ± 0.4	100	14 ± 0.3	100	14 ± 2.4	100
n-hexane-Ethyl acetate	9 ± 1.9	-	8 ± 0.98	-	9 ± 0.55	-	11 ± 0.59	100
Ethanol -n-hexane	8 ± 1.29	-	8 ± 0.75	-	9 ± 0.29	-	9 ± 0.42	-
Methanol-Chloroform	9 ± 1.33	-	6 ± 10.42	33.33	8 ± 0.26	-	14 ± 0.2	100
Methanol-Ethyl acetate	9 ± 2.76	-	8 ± 0.21	-	11 ± 1.41	33.33	9 ± 0.55	-
Methanol -Acetone	-	-	10 ± 0.64	100	10 ± 0.2	100	7 ± 0.73	-
Acetone-Water	10±2.7	100	8 ± 2.1	-	9 ± 0.25	-	14 ± 1.64	100
Methanol-Water	7±0.67	100	7 ± 1.9	33.33	8 ± 0.32	-	9 ± 0.44	-
	***Teucrium stocksianum* root extracts**							
n-hexane	12 ± 0.08	100	9 ± 0.24	-	8 ± 1.66	-	13 ± 0.92	100
Chloroform	12 ± 0.92	33.33	13 ± 0.35	100	6 ± 0.82	-	8 ± 0.43	-
Ethyl acetate	11 ± 0.65	33.33	16 ± 0.12	33.33	8 ± 0.24	-	16 ± 0.78	33.33
Acetone	16± 0.42	3.7	13 ± 0.72	100	10 ± 1.56	33.33	16 ± 1.16	3.7
Ethanol	15 ± 0.66	3.7	11 ± 0.57	100	14 ± 0.31	100	15 ± 1.48	100
Methanol	10 ± 1.32	33.33	15 ± 0.38	11.11	17 ± 0.46	3.7	15 ± 0.94	100
Water	11 ± 0.16	100	14 ± 0.79	100	12 ± 0.21	100	6 ± 0.82	-
n-hexane-Ethyl acetate	9 ± 0.54	-	8 ± 0.68	-	10 ± 0.62	33.33	9 ± 0.25	-
Ethanol -n-hexane	8 ± 1.6	-	9 ± 0.12	-	8 ± 0.16	-	10 ± 0.12	33.33
Methanol-Chloroform	7 ± 0.11	-	12 ± 0.52	100	14 ± 0.84	100	12 ± 0.22	100
Methanol-Ethyl acetate	10 ± 0.84	100	10 ± 0.15	100	8 ± 0.11	-	11 ± 0.45	100
Methanol -Acetone	8 ± 0.98	-	9 ± 1.14	100	9 ± 0.32	-	12 ± 0.23	33.33
Acetone-Water	10 ± 0.2	33.33	8 ± 0.22	-	14 ± 0.90	100	11 ± 0.18	100
Methanol-Water	7 ± 0.13	-	9 ± 1.55	33.33	9 ± 0.78	-	10 ± 0.14	100
DMSO	-	-	-	-	-	-	-	-
Cefixime	25 ± 0.56	3.33	28±0.90	3.33	21 ± 0.73	11.11	23±0.11	3.7

*Zone of inhibition subtracting the diameter of the disc (5 mm). In each disc, the sample concentration was 100 µg (5 µL) in disc diffusion assay. The zone less than 10 mm was considered an inactive fraction. The sign (-) represents no activity at all

Conclusion

Before going to the more advanced and expensive screening methods, it is essential to first screen the extract of the plant with the least expensive and less time-consuming protocols. The authors suggest the best solvents or solvent blends such as ethanol, ethyl acetate, acetone, methanol, and methanol-ethyl acetate (1:1) combination for extraction of secondary metabolite from all three parts of *T. stocksianum* plant. Determination of solvent extraction efficiency and its correlative study with respect to its antioxidant potential is very necessary for cytotoxicity screening. It is concluded that different polarity solvents extract different amounts of phytochemicals, which ultimately have diverse biological activities. The initial screening of this plant in our study indicates that all three parts of *T. stocksianum* have excellent quantities of antioxidant compounds to be utilized for the more advanced cytotoxicity screening using cancer cell lines and identification of the bioactive compounds.

Authors Contribution: IH, AB, LS= Conceptualization, Methodology, Data curation, Formal analysis, Writing-original manuscript, IH, NA= Supervision Validation, Visualization, Software, Formal analysis, Writing-reviewing and editing, ZKS= Supervision, Resources, reviewing and editing.

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