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PHYTOCHEMICAL ANALYSIS OF MEDICINALLY IMPORTANT CONSTITUENTS OF *TEUCRIUM STOCKSIANUM* BOISS

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Abstract

Medicinal plants have an illimitable source of natural compounds for drug development. The study was conducted to authenticate the pharmacological importance of *Teucrium stocksianum* boiss using different biological assays. Dried powder from the leaves of *the Teucrium stocksianum* boiss was used to get crude extract with methanol and the resultant yield crude extract was further partitioned with different solvents in an increasing polarity. The extracts were assessed for phytochemicals, thin layer chromatography, antioxidant, cytotoxic, antitumor, antihemolytic, antimicrobial and antituberculosis activity employing different solvents systems. The solvent fractions were assessed for in-vitro antioxidant assays to compute the quenching ability of H_2O_2 and ethanolic extracts were found more potent to scavenge free radicals (19.2±0.43 µg/ml) as compared to other fractions. The aqueous extracts were found more effective in reducing power assay by showing the highest EC_{50} value of i.e. 5.5±0.81µg/ml. Significant cytotoxicity against brine shrimp was manifested by ethanol (150.62 µg/ml) and chloroform (138.2 µg/ml) extracts while a conspicuous antitumor activity against *Agrobacterium tumefaecins* was exhibited by ethanol extracts (87.9±0.02). A moderate antihemolytic activity was noticeable by ethanol and chloroform extracts. Our study highlights the importance of different solvent extraction procedures in the processing of natural products. It also indicates that this study might be a valuable source of natural antioxidants to obstruct the damage linked to free radicals. Therefore, *T. Stocksianum* boiss could be potentially useful in food and pharmaceutical industry.

Key words: *Teucrium stocksianum* boiss, Anti-oxidant, Cytotoxic, Antimicrobial potential.

Introduction

Medicinal plants have a noteworthy part in upholding the health care condition of human population globally and contribute to the development of natural product based drugs (Zaouali *et al*., 2010). According to the world health organization (WHO), approximately 80% of the world population depends upon natural medicines (Dubey *et al*., 2012). The natural compounds and their active constituents have demonstrated therapeutics perspective as anti-diabetics, anti-inflammatory and antioxidants and other pharmacological properties (Rahmani & Aly, 2015). Most of the organic compounds have definite physiological action on the human body because of their low toxicity and therapeutic performance (Inayatullah *et al*., 2012). The different phytochemicals present in the natural product exhibit strong antioxidant abilities. These antioxidant compounds can be terpenoid, alkaloids, vitamins, minerals (copper, zinc, iodine, selenium, manganese), carotenoid, phenols (Gallic acid, allergic acid, tannins), xanthones, enzymes (catalase, superoxide dismutase, glutathione peroxide) flavonoids (isoflavones, quercetin, catechins, anthocyanins, flavones, epicatechins). These antioxidant compounds have potential to overcome several diseases by keeping the cells from harm caused by reactive oxygen species (Gupta & Sharma, 2006; Govind, 2010). A number of compounds isolated from medicinal plants could be used for the development of new drugs to inhibit the growth of fungal and bacterial pathogens and to quench reactive oxygen species with the possible novel mechanism of action and low cytotoxicity to host cell (Ahmed & Aqil, 2007). The natural compounds having antioxidants and antibacterial activity are important for humans to cure different infectious diseases caused by bacteria (Ahmad *et al*., 2013; Tareen *et al*., 2016; Bilal *et al*., 2017). Prominent examples of drugs acquired from natural products are atropine, digoxin, morphine, aspirin, colchicines, ephedrine, taxol, physostigmine, quinine, vincristine, pilocarpine, vinblastine, tubocurarine and reserpine (Gilani, 2005). Around 10,000 to 53,000 plant species are utilized traditionally, although the number of these natural remedies screened pharmacologically relatively less and appropriate screening is a prerequisite to validate and explore the pharmacological potential of medicinal plants (Sarwat *et al*., 2012)

Pakistan boasts a huge exploited exotic and native plant species which are the potential source for the pharmaceutical industry and future foundation for local inhabitants. Pakistan flora has diverse plants containing around 6,000 distinct varieties. Approximately 80% of the people from rural areas still rely on natural herbs (Mustafa *et al*., 2016). The Lamiaceae family comprises of 4000 species and 220 genera worldwide. They are used as indigenous natural medicines around the globe (Rahim *et al*., 2012). *Teucrium stocksianum* Boiss (TS) belong to family Lamiaceae, endow from the North West territory of Pakistan (Swat, Malakand, Dir, and Hazara). It is known as the perennial savory herb having white, gray leaves and sessile blooms of 10-30 cm height. It grows in shades and high altitude like mountains. The plant leaves

are utilized by local inhabitants as a drug for treating jaundice, cough, diarrhea, abdominal pain and blood purifier (Rahim *et al*., 2012). The extracts were examined for antiulcerogenic (Islam *et al*., 2002), analgesic and anti-inflammatory activities (Radhakrishan *et al*., 2001) and for treatment of diabetes mellitus (Islam *et al*., 2002), epilepsy and hypertension (Ahmed *et al*., 2002). The leaf extract has important phytochemicals such as alkaloids, saponins, anthraquinone, phlobatannins, flavonoids, reducing sugar, tannin, terpenoid and glycoside (Rahim *et al*., 2012). Literature revealed a gap on studying the biological activities of TS using different polar solvents and application of bioassays to explore the pharmacological potential. The present study was carried out to identify the phytochemicals and to examine the efficiency of different solvent extracts for potential antioxidant, cytotoxic, antitumor, antimicrobial, antituberculosis, antihemolytic activities of TS.

Materials and Methods

Chemicals: All Solvents and reagents used in the study were of analytical grade. Sodium dihydrogen phosphate (NaH_2PO_4), Disodium hydrogen phosphate (Na_2HPO_4), Deoxyribose, sodium carbonate (Na_2CO_3), Sodium hydroxide (NaOH) and H_2O_2, Sodium nitrite ($NaNO_2$), Ascorbic acid, Ferric chloride ($FeCl_3$) were purchased from Merck Co.

Plant collection: *Teucrium stocksianum* Boiss (TS) plant samples were collected at the flowering period from the vegetative areas of lower Dir, Malakand Division. The plant leaves were identified by Dr. Rahamtullah Qureshi, Taxonomist, Associate Professor in the Department of Botany, PMAS Arid Agriculture University, Rawalpindi, Pakistan.

The voucher specimen (TSL9001) was maintained in the Herbarium of Department of Botany, PMAS Arid Agriculture University, Pakistan.

Extract preparation: The TS leaves were washed with distilled water to rid of dust particles and then dried under shade for 1 week. To prepare crude of dried leaf samples, Willy machine of 60 mesh size was utilized and the afterward coarse powder was used for solvent extraction. 200 g of fine leaf powder was extracted with 1 liters of methanol at 37°C for 72 hours using an electrical orbital shaker. Solvent from the samples was filtered and evaporated off under reduced pressure in a rotary evaporator to obtain the crude methanol extract.

Fractionation of extract: The crude extract was divided into two parts. One part of the extract (20 g) was assayed as crude methanolic extract and the remaining part (80 g) was partitioned with various solvents. The 80 g of extract was dissolved in 500 ml of d.H_2O and subjected to the separatory funnel for liquid partitioning. Almost 400 ml of n-hexane was dissolved and the upper layer of n-hexane was collected and the remaining aqueous layer was re-partitioned with fresh n-hexane. The whole process was performed in triplicate. All the n-hexane fractions were pooled to gather, filtered and dried by using a rotary evaporator. A similar procedure of partitioning was carried for other solvents based on order of polarity: chloroform, butanol and ethanol. After completing the whole procedure, the remaining lower aqueous layer was gathered and dried. All the fractions were kept at 4ºC.

Percent extracts yield: The dried extracts of plants were weighted and the percentage yield of the extract was calculated by following formula;

$$\% \text{ Yield} = \frac{A}{B} \times 100$$

A is the total weight of crude plant extract and B is the total weight of the dried powder plant.

Phytochemical analysis: A dried leaves powder was utilized for qualitative phytochemicals test in order to check the presence of flavonoids (Trease & Evan, 1996), phenols and tannins (Roopashree *et al*., 2008), alkaloids (Tyler, 1994), saponins (Hussain *et al*., 2011) steroids, phlobatannins, glycosides, terpenoids and anthraquinone according to procedures previously illustrated with slight modifications (Harbone, 1998).

Mineral analysis: The total mineral analysis was performed following AOAC Method No. 930.0. 1 gram of sample was taken in the porcelain crucible and subjected to dry ash mineralization at 450ºC. The obtained residue was dispersed in 1 ml of conc. H_2SO_4, 1 ml of H_2O_2 and 1 ml of HNO_3 and then dilute with deionized water to make the volume up to 10 ml. The amount of minerals Zn, Mg, Mn, Ca, Fe, Cd, Cr, Pb, and Cu were detected using Atomic Absorption Spectrophotometer (AAS) with flame in the Analyst and comparing the absorbance with analytical standard solutions for AAS, supplied by Merck, Germany (Ruiz-Rodríguez *et al*., 2011).

Thin layer chromatography fingerprints: TLC was carried out following the procedure of (Banu & Nagarajan, 2014).10 mg of plant samples was dissolved in 1 ml of respective solvents. The plant samples 10 µl were applied to the TLC plate by using a glass capillary tube (TLC grade, Merck Germany). The spotted TLC plates were put into the chromatographic chamber. The solvent system having different polarities i.e. Chloroform: Acetone (8:4 and 6:4 v/v), Chloroform: Methanol (1:9 v/v), Toluene: Ethyl acetate: Formic acid (3:1:1) are utilized to develop system and air dried the plates. The iodine and H_2SO_4 vapors were sprayed to TLC plates and placed the plate on the hot plate for 2 minutes. The colored spot was identified in the plates. The plates were air dried and visualized the spot under ultraviolet (UV) light at 254 and 365 nm. The distance of the samples spot was expressed by its retention factor (R*f*). The R*f* value of the observed spot was calculated using the following formula:

$$Rf = \frac{\text{Distance travel by sample}}{\text{Distance travel by solvent}}$$

Antioxidant assay

Reducing power assay: The reducing power or FRAP (Ferric ion reducing power) was measured according to the method of (Oyaizu, 1986). Different concentration of test sample ranging (20-100 µg/ml), 2 ml potassium ferricyanide (10 mg/ml), 2 ml phosphate buffer (0.2 M, pH 7.4) was added, and then incubated for 20 min at 50°C, further 2 ml of trichloroacetic acid (TCA, 10%) was added to the mixture centrifuged at 3,000 rpm for 10 minutes. The upper layer of the solution was mixed with 0.5 ml ferric chloride (1% w/v). Incubate the reaction mixture for 10 minutes, the readings of blank and samples were observed at 700 nm. Ascorbic acid was used as positive control. The results were expressed as GAE (mg/g of extract).

Hydrogen peroxide scavenging assay: The hydrogen peroxide scavenging activity of the samples was performed through the procedure of (Ruch *et al*., 1989). A 2 mM solution of the H_2O_2 was prepared in 50 mM phosphate buffer (pH 7.4). Different concentration of extracts was added into the test tubes and their volumes were made up to 4 ml with the addition of H_2O_2 solution (pH 7.4). Subsequently, test tubes were vortex and incubate for 10 minutes. The absorbance of H_2O_2 was determined at 230 nm against blank solution containing phosphate buffer without hydrogen peroxide and compared with the ascorbic acid used as a standard drug.

The capacity to scavenge the H_20_2 was determined using the following formula:

$$H_2O_2 \text{ scavenging } \% = \frac{\text{1-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Cytotoxic brine shrimp assay: The cytotoxicity of TS and its fractions was carried out through the procedure of Ahmed *et al*., (2009). The *Artemia Salina* (brine shrimp) was used as test organisms. 10 mg of TS and each fraction was dissolved in 1 ml of DMSO. The further dilutions of (50-1000 µg/ml) were prepared and solvents were used as negative control. Etoposide (7.4 µg/ml) standard drug was used as positive control.

The % inhibition of TS fractions was calculated using the following formula:

$$\text{Death } (\%) = \frac{\text{Test sample - Control}}{\text{Control}} \times 100$$

Antitumor potato disc assay: The potato disc assay was used to evaluate the anticancer activity of plant extracts by the procedure of (Ferrigini *et al*., 1982). The virulent strain of *Agrobacterium tumefaciens* (AT10) was grown in Lauria broth (LB) media, including rifampicin (10 µg/ml) for 48 hours. Fresh red skinned potatoes were soaked in 0.1% mercuric chloride for 10 minutes. Sterilized borer was used to cut 5 mm thick disc of potatoes and placed in agar (2%) plates in triplicate. Agrobacterium culture (1 ml) mixed with 40 µl extract concentrations of 10, 100, 500, 1000 µg/ml (dissolved in DMSO) was added to the surface of each potato disc of particular concentration. Petri plates were placed in the incubator for 21 days at 28ºC. After 21 days, Lugol's solution (10 % of KI and 5 % of I_2) was used to stain the discs for 30 minutes and then observed under a microscope. The portion of the disc remains unstained was tumor. The following formula was used to count the number of tumors per disc:

$$\% \text{ Inhibition} = \frac{100 - (\text{No of tumor in plant sample})}{(\text{No of tumor in negative control})} \times 100$$

Antihemolytic activity: The method is according to the method of Yang *et al*., (2005) with slight modification. The TS solvent fractions were assessed against Human erythrocytes cells. The absorbance of the test sample was measured by using UV Spectrophotometer at 540nm.

$$\% \text{ Antihemolytic activity} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Antibacterial activity: Plant samples were screened for determining antibacterial potential by agar well diffusion assay against four bacterial strains, *Staphylococcus aureus* (ATCC6538), *Escherichia coli* (ATCC15224), *Klebsiella pneumonia* (MTCC618) and *Bacillus subtilis* (ATCC6633). Whereas standard antibiotics (Ciprofloxacin) was used for comparison and absorbance was determined at 420 nm by using spectrophotometer (Ettebong & Nwafor, 2009). The minimum inhibitory concentration was estimated on the basis of the lowest concentration of the extracts that blocked the further progress of bacteria after 24 hours of incubation period.

Anti-tubercular activity: The two drug-resistant strains of *Mycobacterium tuberculosis*, bg 206 and bg 1972, while a sensitive strain H37Rv was used in the current study. The indigenous "bg 206" strain was resistant to streptomycin, rifampicin and kanamycin while another indigenous mycobacterial strain"bg 1972" was resistant to streptomycin, Isoniazid, Rifampicin, Ethambutol and Ofloxacin. Reference strain H37Rv was sensitive to all anti-tuberculosis drugs. WHO recommended Lowenstein-Jensen (LJ) medium for growth of Mycobacterium species was used (Gemechu*et al*., 2013). All these strains were obtained from National TB Reference Laboratory, National TB Control Program Chak Shahzad, Islamabad.

Statistical analysis

All the results were expressed as mean ± SD. Statistical analysis was performed using SPSS 15. The significant difference was considered at ≤ 0.05.

Results and Discussion:

Extraction yield: The pattern of extract yield was as follows methanol > ethanol > butanol > aqueous > chloroform > n-hexane. The percent yield of these solvent extracts varied from 50.4±0.50 % to 5.0±0.75 % as shown in table 1. Extraction with methanol, ethanol, butanol result in the remarkable quantity of total extractable components, however, the extraction with aqueous, chloroform, and n-hexane was significantly less (p<0.01) in comparison with remaining extracts.

Table 1. Color, consistency and % yield of TS extracts.

	Extracts	Color	Consistency	Crude weight	% Yield
1.	n-hexane	Brown	Semisolid, non-sticky	5.0 ± 0.03g	5.0%
2.	Chloroform	Yellowish	Powder	10.8 ± 0.56g	10.8%
3.	Butanol	Dark brown	Sticky powder	20.8 ± 1.02g	20.8%
4.	Ethanol	Blackish brown	Semisolid and sticky	30.2 ± 0.97g	30.2%
5.	Methanol	Yellow brown	Resinous	100.4 ± 0.07g	50.4%
6.	Aqueous	Light Yellow	Powder	15.5 ± 0.24g	15.5%

Table 1. Qualitative determination of phytochemicals from TS extracts.

	Phytochemicals	Methanol	Ethanol	Chloroform	Butanol	Aqueous	n-hexane
1.	Phenols	+	+	+	+	+	+
2.	Alkaloids	+	+	+	+	+	+
3.	Glycosides	+	-	-	-	+	-
4.	Flavonoid	+	+	+	+	+	+
5.	Terpenoid	+	+	+	+	+	+
6.	Tannins	+	+	+	+	+	+
7.	Phlobutanins	-	-	-	-	-	-
8.	Saponin	+	+	+	+	+	+
9.	Steriod	+	+	-	-	+	-
10.	Anthraquinone	-	+	+	+	+	+

Mean values ± standard deviation, after triplicate analysis

Qualitative phytochemical analysis: Phytochemical analysis is carried out to study novel and valuable compounds with bioactivities. The phytochemicals screening of ethanol, methanol, chloroform, n-hexane, butanol and d.H_2O extracts of *T. stocksianum* boiss showed the presence of medicinal bioactive compounds (Table 2). Among these, flavonoids, alkaloids, phenols, tannins terpenoids and saponins were present in all extracts whereas glycosides are present in methanolic and aqueous extracts. Flavonoids are pharmacological important for the production of anti-allergic, anti-inflammatory and anti-microbial medicines (Kumar *et al.*, 2011). Anthraquinone was found in all extracts except methanol extract, Phlobatannins was absent in all solvent extracts. The results acquired in this study implied that identified phytochemicals in plant extracts are important reservoirs of potent bioactive compounds. Secondary metabolites such as phenolics, flavonoids and proanthocyanidins have shown a correlation with antioxidant activities (Afshar *et al.*, 2012). Rahim *et al.*, (2012) also reported alkaloids, saponins, anthraquinone, phlobatannin, flavonoids, reducing sugar, tannin, terpenoid and glycoside in the leaf extracts of TS.

Mineral analysis: The *T. Stocksianum* boiss was also evaluated for important elements through an atomic absorption spectrophotometer. The CD was present in 0.094 ppm, which shown that TS extracts have low Cd content than the permissible limits of FAO or WHO. A similar study was reported by Sindhu & Beena (2016) they reported the presence of Cd below detection levels in the medicinal plants. However, the concentration of Zn was significant higher (0.757 ppm). Zn is most important trace element present in the bones and muscles (85%), 11% in the liver and the skin, whereas the rest is distributed in all other parts of the body (Tapiero & Tew, 2003). Nickle and iron were found higher 0.558 ppm and 0.472 ppm respectively. The mineral analysis revealed a significant amount in the TS extracts. In the human body, mineral elements play a potent role as curative and preventative agents against different diseases (Shahban *et al.*, 2016).

Thin layer chromatography: TLC fingerprinting of TS extract gives impressive output towards the presence of different bioactive compounds. Separation of different polar compounds varies with the different combination of organic solvents as evident from the observation. Chloroform and acetone (8:4) yield high resolution secondary metabolites such as flavonoids and phenols. Precise separation of compounds was witnessed by methanol and ethanol extracts. The quality of resolution between the compounds was differing based on their solvent polarity and retention time. The 5 compounds were identified by a methanolic fraction with R*f* value of 0.25, 0.36, 0.42, 0.53, 0.68. The 4 compounds were revealed by ethanol (R*f* 0.45, 0.55, 0.59, 0.71) and butanol (*Rf* 0.14, 0.29, 0.43, 0.55) fractions. TLC is an important technique for the quality control of the herbal product, but also for the analysis of natural drugs (Duron *et al.*, 2009). It is an important method to analyze plant-based drugs and used for different applications. In various pharmacopeias, a vast variety of plant phytochemicals were identified by using TLC fingerprinting (Renato *et al.*, 2012). The appearance of different compounds and bands can be valuable for the authentication and identification of plant drugs.

Table 3. Antioxidant effect (EC_{50}) on H_2O_2 scavenging activity and Reducing power assay of different soluble extracts of TS extracts.

Plant extracts	EC_{50} µg/ml	
	H_2O_2 scavenging activity	Reducing power assay
n-Hexane	87.2 ± 0.47	15.7 ± 0.43
Chloroform	45.1 ± 2.75	22.9 ± 0.95
Butanol	51.6 ± 0.05	20.5 ± 0.39
Ethanol	34.6 ± 0.33	34.2 ± 0.04
Methanol	18.8 ± 1.02	7.7 ± 1.25
Aqueous	12.8 ± 0.17	5.5 ± 0.81
Ascorbic acid	16.9 ± 0.37	6.5 ± 0.36

Each scale value shown as mean (n=3) ± SD. Probability level in each segment LSD at $p<0.01$

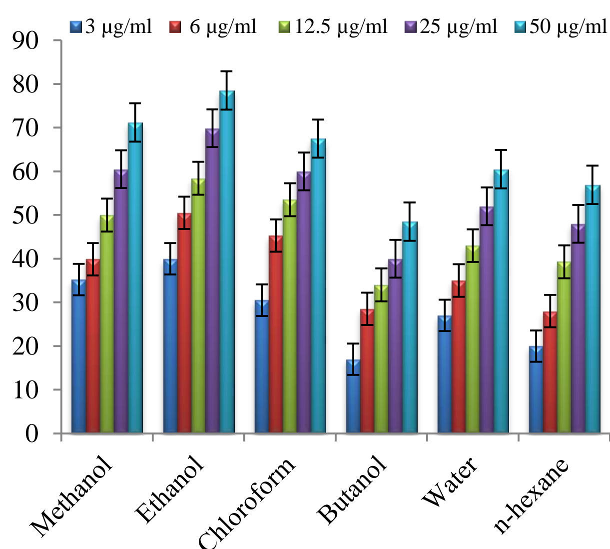

Fig. 1. Brine shrimp assay for the determination of cytotoxicity of *T. stocksianum* boiss extracts.

Antioxidant assay: TS extracts possessed strong scavenging activity against H_2O_2 radicals. The highest EC_{50} value was obtained for TS ethanol extract (17.5±0.05 µg/ml), the EC_{50} of ethanol extract is nearby to standard ascorbic acid (16.4±0.89 µg/ml) at 100 µg/ml ($p<0.05$). The radicals reducing the potential of TS extracts ranges from 17.5±0.05 to 37.8±2.35 µg/ml (Table 3). The TS ethanolic extract showed a significant increase ($p<0.05$) with respect to other TS extracts. Phenols are an important class of antioxidants due to their ideal structural chemistry for free-radical scavenging activity. They can act as reducing agents, metal chelators and free radical quenchers by donating an electron or hydrogen atom to free radicals (Maqsood *et al*., 2017). Phenolic compounds act as primary antioxidants which have an ability to quench free radicals that inhibit initiation or disrupt propagation of lipid oxidation, by decreasing the generation of decomposed products e.g ketones and aldehydes that rancidity (Shahidi *et al*., 2015). The methanolic extract possesses high phenolic content (79.2± 1.27 mg GAE/g). The genus *Teucrium* different species are rich in phenolic compounds and has shown strong biological activities (Hasani-Ranjbar *et al*., 2010).

In reducing power activity plants possess compounds that act electron donors which have the ability to work as primary and secondary antioxidants. Reducing the power of a compound may serve as a significant indicator of its potent antioxidant ability (Hameed *et al*., 2012). The sequence for reductive ability for TS leaves extracts were as follow aqueous > methanol > n-hexane > butanol > chloroform > ethanol fractions. The aqueous extract possessed the highest reducing capacity followed by the ethanol extract. The EC_{50} value of aqueous extract (5.5±0.81 µg/ml) was comparatively more potent than ascorbic acid (6.5±0.36 µg/ml) which was used as positive control. All the solvent extract show good reductive power in a series of concentration-dependent pattern ($p<0.01$). The polarity based antioxidant activities are in accordance to previous studies reported by Stankovic *et al*., (2011) and Shah & Shah (2015).

Brine shrimp lethality assay: The cytotoxic potential of *Teucrium stocksianum* boiss was evaluated using nauplii of brine shrimp. The larvae of shrimps are behaved analogous to mammalian carcinoma cells and toxic effect of tested samples might demonstrate their possible antitumor and anticancer activity (Ullah *et al*., 2012). The extracts have demonstrated remarkable cytotoxicity and ethanol extract proved to be more potent with LD_{50} of 150.62 µg/ml. The TS ethanol extract showed the highest lethality against shrimps however, the mortality rate was found to be different at various concentrations (50-1000 µg/ml). Three fractions of TS including ethanol, methanol and chloroform, showed the highest death rate of shrimps in comparison with rest of the extracts. Results of brine shrimp cytotoxicity are shown in Fig. 1. The results showed that cytotoxicity was concentration-dependent manner and decreased in sample concentration were decreased in percent mortality. Results are according to previous reported studied (Khan *et al*., 2015). The cytotoxicity of extracts might be due to the presence of active secondary metabolites such as flavonoids, alkaloids and phenols (Aziz *et al*., 2013). Shah *et al*., (2014) demonstrated that TS extract has strong anticancer activity. The saponin extract of TS showed potent cytotoxic effect (LC_{50}<10 µg/ml) against brine shrimps.

Potato Disc Antitumor assay: The extracts showed significant cytotoxicity were subjected to potato disc assay to validate the anticancer potential. The strain of *Agrobacterium tumefaciens* was used against different concentrations of (10, 100, 500, 1000 µg/ml). The TS ethanolic (87.9±0.01) and TS chloroform (75.4±0.35) extract possess the highest tumor inhibition activity (Table 4). Results were compared to positive control vincristine sulfate, which proved anticancer nature of extracts. The principle behind the potato disc was to acquire rapid, reliable and economic outcomes. The *A. tumefaciens* induced tumor relies on potato tumor can express the 3PS activity in comparison to 9PS (Ahmed *et al*., 2016). The different extracts of TS had shown significant tumor inhibition in disc models.

Table 4. Antitumor potential of different extracts of TS against potato disc assay.

Extracts	10 µg/ml	% Tumor 100 µg/ml	Inhibition 500 µg/ml	1000 µg/ml
n-hexane	2.4±3.05	11.9±0.29	23.6±0.37	35.9±2.05
Chloroform	31.4±0.62	46.8±0.05	60.3±2.37	75.4±0.35
Butanol	14.5±1.58	28.8±0.65	48.4±0.92	62.2±0.72
Ethanol	39.4±0.62	58.2±0.79	72.6±0.08	87.9±0.02
Methanol	6.6±2.07	17.2±0.09	29.4±0.29	40.1±0.12
Aqueous	14.4±0.49	22.5±0.58	34.7±0.83	48.6±1.51

Value represented as mean of ± SD (n=3). Each column has significant different LSD at (p<0.01) probability level and their mean not sharing same letter

Table 5. Antibacterial activity and minimum inhibitory concentration (MIC) of TS extracts against gram positive and negative strains.

n-hexane	Extracts							
	S. auresu	MIC	*E.coli*	MIC	*B.subtilis*	MIC	*K. pneumonis*	
Chloroform	20.4±0.55	2.89	13.5±0.65	100	14 ± 0.23	33.33	22.5±1.55	100
Butanol	12.5±0.35	100	7.8±0.45	2.5	8.5±0.55	-	10.5±0.90	-
Ethanol	14.5±0.25	2.21	11.2±0.24	33	10.8±0.85	-	12 ± 2.22	33.33
Methanol	18.7±1.72	2.51	8.4±0.19	-	12.5±1.75	100	18.3±1.25	-
Aqueous	8.4±2.85	-	7.9±1.55	10	8.5±0.01	-	14 ± 1.54	33.33

The concentration of sample was 100 µg per disc. Mean ±SD (n=3). Ciprofloxacin as positive control and DMSO as negative control. Diameter of zone of inhibition in mm (±SD) MIC µg/ml

Table 6. Anti-tuberculosis activity of TS extracts.

Extracts	Isolates	Mean CFU on media				Percentage Inhibition		
		Control	5mg/ml	10mg/ml	50mg/ml	5mg/ml	10mg/ml	50mg/ml
Chloroform	H37Rv	140	20	0	0	86	100	100
	bg 206	150	42	15	0	73	90	100
	bg 1972	130	71	40	0	46	69	100
Ethanol	H37Rv	140	35	0	0	79	100	100
	bg 206	150	45	20	0	70	87	100
	bg 1972	130	65	50	0	50	62	100

Percentage inhibition = Cc – Ct/Cc x100, Cc = No of colony in the control media slope, Ct = No colony in the Test media slope

Antihemolytic activity: The effect of TS extracts on human RBCs had shown that they did not demonstrate any harmful consequence on human RBCs. TS extracts illustrate differential hemolytic pattern towards erythrocytes and exhibit good inhibitory activity at low concentrations. TS methanol extract inhibited hemolysis of cells as maximum by showing 80.5±0.02% at the concentration of the 75 µg/ml. The TS extracts have shown that by increasing the concentration of extracts steadily hinder hemolysis of erythrocytes; hence the protective effect of TS can be due to RBCs protection, which put off the peroxidation of membrane and hemolysis of red blood cells.

Antibacterial activity: The antimicrobial activity of medicinal plants depends upon solvents, the part of the plant and tested organism (Azam *et al*., 2017). *T. stocksianum* has demonstrated the folklore use and secondary metabolites have indicated the presence of novel compounds likes flavonoids and phenolics. The antimicrobial activity was determined by using agar well method. Extracts with the higher zone of inhibition >12 mm were further investigated at low concentration and their MICs were estimated using the broth microdilution method. *Staphylococcus auresu* was found more sensitive with chloroform extracts having a zone of inhibition of 20.4±0.55 mm and MIC value of 2.89 µg/ml followed by methanol extracts with 18.7±1.72 mm inhibitory zone and 2.51 µg/ ml MIC value. Significant results were observed in case of *Klebsiella pneumonia* with aqueous extracts showing maximum activity having a zone of inhibition equal to 14±1.54 mm and MIC of 33.33 µg/ml. It was followed by ethanol with 12±2.22 mm zone of inhibition and 33.33 µg/ml MIC value. Moderate activity was noted *against Bacillus subtilis* with largest inhibitory zone of 14±0.23 mm observed with n-Hexane extract and a resultant MIC of 33.33 µg/ml (Table 5). No activity was observed with negative control (DMSO) which confirmed its non-toxic effect. *Teucrium* species have shown remarkable antimicrobial activity against different human pathogens (Vukovic *et al*., 2011). Shah *et al*., (2015) has demonstrated profound antibacterial activity of TS extract against *E. coli* (46%), *S. aureus* (30.3%), *S. Type*

(68.6%) these results are in accordance with our reported results. Ahmed *et al*., (2008) reported that *T. royleanum* solvent extracts demonstrated 100% inhibition against *S. typhi* bacterial strain.

Anti-tuberculosis activity: The aqueous and n-hexane extracts did not show activity against tuberculosis strains used in the study. They were used in three different concentrations i.e., 0.5mg/ml, 10mg/ml and 50mg/ml. There was no inhibition of growth of strain H37Rv in the slopes containing extracts at the different concentration as observed in control (Table 6). The ethanol and chloroform extracts showed inhibition at different concentration against the H37Rv strain.

Conclusion

The detailed screening of TS extracts depends upon different solvents and their polarity. The detection of important phytochemicals in the extracts may demonstrate the reported measures and traditional uses. In the present study ethanol and chloroform extracts have a potential source of phytochemicals provoking noteworthy antioxidant activities. The plant extracts have shown significant antibacterial activities against selected bacterial strains. Cytotoxicity assay confirms that ethanol extract is highly efficient against brine shrimp, moreover, ethanol and chloroform extracts showed remarkable antitumor potential against *Agrobacterium tumefaciens*. Our study confirmed the pharmacological potential of *T. Stocksianum* boiss based on prominent outcomes of multiple assays.

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