PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTI-MICROBIAL ACTIVITY OF *TEUCRIUM OLIVERIANUM* GING EX BENTH.

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ABSTRACT

Teucrium oliverianum Ging ex Benth. is used in Mediterranean region as antidiabetic, analgesic and anti-inflammatory remedy. Literature search revealed that much work has been done on other species of genus *Teucrium* but very little research work is carried out on *T. oliverianum.* We screened it for phyto-constituents and antimicrobial activity. Seventy gram crude extract was prepared from whole plant using classical method and fractionated with organic solvents (*n*-hexane, chloroform, ethyl acetate and *n*-butanol) yielding 22g, 17g, 11g and 20g of each fraction respectively. Phytochemical analysis was done on the fractions using standard method. Antibacterial activity was investigated using agar well diffusion procedure against some pathogenic gram positive, gram negative bacterial and fungal strain *Candida albicans*. Phytochemical studies showed positive results for alkaloids, carbohydrates/glycosides, tannins and triterpenes/sterols and absence of Anthraquinones in all fractions. Saponins were present in hexane and ethyl acetate fractions while flavonoids were absent in *n*-butanol fraction only. Mild significant antibacterial activity was found in Chloroform fraction. Ethyl acetate and n-Butanolsamples exhibited high activity against *E.coli*. Antifungal activity was found in Hexane and Ethyl acetate fractions.HPLC fingerprinting of *Teucrium oliverianum* ethanol extract was performed which showed small peaks starting from 17thminute and major peaks at 31st and 32nd minute which can be used for identification of this plant.Thus in short, the study revealed that different fractions contain a number of active phyto-constituents and mild significant antibacterial and antifungal activity which may be useful for further exploration.

Key-words: *Teucrium oliverianum* ;phyto-constituents; antimicrobial; HPLC fingerprinting

INTRODUCTION

Teucrium oliverianum Ging ex Benth is a perennial shrub belonging to family Lamiaceae. It is grown in silty soils of inland basins and rocky runnels covered with thin sand layers. It is found in Northern Border province and nearby areas (Abu Asab and Cantino, 1993; Al Hemaid, 1998).Genus *Teucrium* contains almost 340 species. Literature search shows anti-oxidant, analgesic, anti-inflammatory, anti-ulcer, insecticidal, anthelmintic, cytotoxic, antispasmodic, antibacterial and antifungal activities in various species viz.*T. polium, T. orientale, T. royleanum, T. buxifolium and T. stocksianum*(Ahmad *et al*, 2008; Fernandez *et al*., 1997; Gharaibe *et al*, 1998; Shah *et al*, 2012; Yildrim*et al*, 2004; Ahmadi and Shahmir, 2002). *T. polium* is reported for its antioxidant and xanthine oxidase inhibition activities (Alkofahi and Atta, 1999; Ljubuncic *et al*., 2006). Hydro-alcoholic extract of this plant exhibited mild analgesic effect andthe author suggested mode of action through opioid receptors, stimulation of GABAergic system, promotion of endogenous opipeptides release or decrease of free radicles (Arzi *et al*., 2011).*T. monatum* and *T. divaricatum* also possess diterpenoids as reported by some authors (Malakov*et al*., 1978; Bruno *et al*., 1987).

In Kingdom of Saudi Arabia *Teucrium oliverianum* is used for diabetes as a traditional medicine (Zechmeister, 1994).The species is previously reported to possess active compounds like Tecrolivin A, Teucrolivin B, Teucrolivin C and Teucrolivin H (Zechmeister, 1994; Bruno *et al*., 2004). Diterpenoids are reported in aerial parts of *T. oliverianum* (Al-Yahya*et al*., 2002). Other species of Genus *Teucrium* contains components like monoterpenes, diterpenes, sterols, Iridoids, alkaloids, Flavonoids, Saponins, Polyphenols and Volatile oil (Arzi*et al*., 2011). The major components of essential oil in this genus include δ-cadinene, α-pinene, myrcene, β-caryophyllene, germacrene D and limonene (Gharaibe *et al*., 1998).

Literature search revealed that much work has been done on other species of genus*Teucrium* but very little research work is carried out on *T. oliverianum* (Fatima, 2016). So it was decided to screen the above mentioned species for phyto-constituents and antimicrobial activity.

MATERIALS AND METHODS

Extract Preparation

Air–dried powdered of whole plant *Teucrium oliverianum* (500 g) was subjected to percolation with 10 folds ethanol 70%. The combined alcoholic extracts were concentrated under reduced pressure at a temperature 40^{o}C till dryness to yield (70 g) of total extract. The concentrated ethanol extract was mixed with 0.5L of deionized water and partitioned several times with *n*-hexane (A), chloroform (B), ethyl acetate (C) and *n*-butanol (D), then concentrated under reduced pressure at 40^{o}C to give 22, 17, 11, 20g,respectively using classical method (Fatima, 2009)**.**

Phytochemical Studies

Phytochemical studies were carried out on all fractions by reported method. The tests were performed to find out the presence of Alkaloids (Mayer's Test, Wagner's Test and Dragendorff's Test), Carbohydrates/Glycosides (Molisch Test, Fehling Test), Tannins ($FeCl_3$ Test), Saponins (Froth Test), Flavonoids (Sodium Hydroxide Test), Triterpenoids (Acetic anhydride Test) and Anthraquinones (Borntragers Test). All chemicals used were of analytical grade (Pochapski*et al*., 2011; Evans, 2009).

HPLC fingerprints of extract

The determination of the chromatograms was carried out for *Teucrium oliverianum* extract by HPLC using various solvent systems as mobile phase. Analysis of extract was performed using RP-HPLC, Waters® 2545 Quaternary Gradient Module pump and equipped with Waters® 2998 diode array detector, and chromatograms were obtained between 210-400 nm. This entire system was controlled using Empower 3 Software. The column used in the system is water guard with asymmetry C18 of 5µm, 4.6*250mm dimensions and 20 µL volume of sample was injected using Hamilton syringe.Two solvent systems A consisting of Methanol and B consisting of Formic acid and distilled water were used as gradient elution in RP-HPLC method. The detector was adjusted between 210 -400 nanometer wavelengths with a flow rate of 1.4 mL/min. The program was run with 25% A in the beginning and this concentration was kept hold for initial five minutes. Then, it was followed by 100%A with holding time 0.5 h (Abdelwahab MF *et al*., 2016).

Antimicrobial Activity

Agar well diffusion method was used to investigate Antimicrobial activity following reported procedure (Jawad *et al*, 1988; Valgas *et al*, 2007). The test sample was used in concentration of 20mg/mL and zones of inhibition were measured mm ± standard deviation. The activity of tested samples was studied against the *Staphylococcus aureus* (AICC25923) and *Bacillus subtilis* (NCTC8236) while *Pseudomonas aeruginosa* (AICC27853), *Escherichia coli* (AICC25922), and *Candida albicans* (AICC7596).

RESULTS AND DISCUSSION

The initial phytochemical screening of hexane, ethylacetate, chloroform and n-butanol fractions of *Teucrium oliverianum* ethanol extract revealed that all fractions contain alkaloids, carbohydrates, tannins and triterpenes/sterols while Anthraquinones were not found in any fraction. Saponins were found in hexane and ethyl acetate fractions while flavonoids were absent in *n*-butanol fraction only (Table 1). The constituents such as alkaloids, carbohydrates, tannins, flavonoids, anthraquinones have curative activity against various ailments including certain pathogenic organisms which justifies its use as a traditional medicine.

Anti microbial activity of all four fractions i.e. (A), (B), (C) and (D) fractions was carried out against some bacterial and fungal strains mentioned in Table2. If 0.2mg/mL concentration of test sample produce 6 mm zone against clinically pathogenic organism then it is considered useful (Abdel-Khaliq, 2014). All fractions except fraction (A) were found effective for *E. coli*, while all were ineffective for *P. aeruginosa*. The zones of inhibition measured 0mm, 12.0± 0.10mm, 14.0± 0.50mm , and 10.0± 0.20mm respectively against (A), (B), (C) and (D) fractions. The standard drug Amikacin formed 19.0± 0.30mm of zone against *E. coli* and 20.0± 0.10mm against *P. aeruginosa*. Both of the above mentioned organisms are Gram negative but the result suggests its selective use for *E. coli* infection. As far as Gram positive organisms are concerned, mild antibacterial activity was found in Hexane fraction only i.e. 6.60± 0.630mm zone inhibition against *S. aureus* and 9.40± 0.440mm against *B. Subtilis*, while the standard drug Carbenicillin formed 23.80± 0.20mm and 32.40± 0.30mm zones of inhibition, respectively.

Antifungal activity was screened for *Candida albicans* which exhibited mild activity. The size of zones was found to be 10.60± 0.030mm, 0mm, 9.60± 0.600mm and 0mm against (A), (B), (C) and (D) fractions respectively. The standard drug Amphotericin B formed 25.40± 0.100mm zone of inhibition against *C. albicans.*

Table 1.Screening of Phyto-constituents in *Teucrium oliverianum.*

Phyto Constituents	Fraction A	Fraction B	Fraction C	Fraction D
Alkaloids	Present	Present	Present	Present
Carbohydrates	Present	Present	Present	Present
Tannins	Present	Present	Present	Present
Saponins	Present	Present	Absent	Absent
Flavonoids	Present	Present	Present	Absent
Triterpenes	Present	Present	Present	Present
Anthraquinones	Absent	Absent	Absent	Absent

Table2. Antimicrobial activity of plant extracts against selected microorganisms.

Tested microorganisms	Fraction (A)	Fraction (B)	Fraction (C)	Fraction (D)	Standard
Fungi	Diameter of inhibition zone (mm)				Amphotericin B
Candida albicans(ATCC7596)	10.60± 0.030	0.0	9.60± 0.60	0.0	25.40± 0.100
Gram +ve bacterialstrains					Carbenicillin
Staphylococcus aureus(ATCC25923)	0.0	6.60± 0.630	0.0	0.0	23.80± 0.200
Bacillus subtilis (NCTC8236)	0.0	9.40± 0.440	0.0	0.0	32.40± 0.300
Gram -ve bacterial strains					Amikacin
Pseudomonas aeruginosa (ATCC27853)	0.0	0.0	0.0	0.0	20.0± 0.10
Escherichia coli (ATCC25922)	0.0	12.0± 0.10	14.0± 0.50	10.0± 0.20	19.0± 0.30

Unit test strains were of Regional Center for Mycology and Biotechnology Antimicrobial Test OrganismsData as mean ± SD**.**

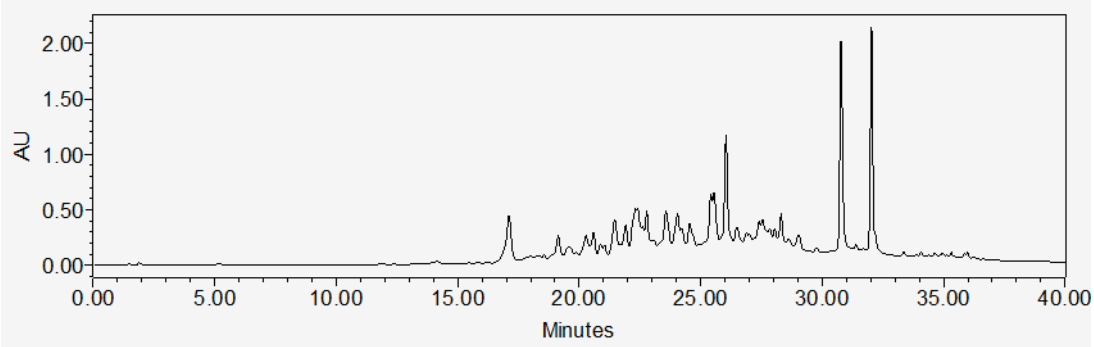

Fig.1.HPLC fingerprints of*Teucrium oliverianum.*

Hexane (A) fraction had activity against only *C. albicans.* Chloroform (B) fraction showed better activity against *S. aureus*, *B. subtilis* and *E. coli*. Ethyl acetate (C) fraction exhibited activity against *Candida albicans* and *Escherichia coli.n*-Butanol (D) fraction showed activity only against *Escherichia coli.*

HPLC finger printing of *Teucrium oliverianum* ethanol extract was performed which showed small peaks starting from 17th minute up to 30th minute run. The two major peaks were foundat 31st and 32nd minute. HPLC fingerprinting of *Teucrium oliverianum* ethanol was conducted to develop a pattern which will be useful for identification of herbalmaterial as well as help in screening of pharmacologically active component which is usually found in minute quantity in crude extract. Further studies can be carried out to separate the component and test for pharmacological activities.

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