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In vitro phytochemical and antimicrobial screening of *Thymus linearis*

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Abstract

Various solvent extracts from the whole plant of *Thymus linearis* were screened for their phytochemical and antimicrobial potentials. Preliminary phytochemi-cal screening of plant extracts showed the existence of terpenoids, flavonoids, tannins, alkaloids, glycosides and reducing sugars. Fourier transform infrared spectroscopy studies were carried out on various phytochemicals extracted from the exhibited *T. linearis* which resulted in the presence of different compounds like amides, aldehydes, carboxylic acid, ether, alcohol and ketones. All the extracts of *T. linearis* showed significant antibacterial and antifungal activities when tested against nine bacterial and four fungal strains. It was concluded from this study that extracts of *T. linearis* have an array of important phytochemicals and significant activities against some of the multidrug resistant bacterial and medically important

Introduction

The global emergence of bacterial resistance is responsible for many infectious diseases (Westh et al., 2004). Although many antibiotics have been introduced, the drug resistance in bacteria has continuously escalated (Ishaq et al., 2014; Hussain et al., 2014). This situation directed the interest of researchers towards the development of novel drugs from herbal products, having antimicrobial potentials (Maiyo et al., 2010).

From ages mankind exposed the subsistence of microbes to plants with the idea that certain plant had therapeutic potential. Certainly, these plants contained what we would presently exemplify as anti-microbial principles (Rios and Recio, 2005).

Thymus linearis, an imperative medicinal and food source belongs to the family Lamiaceae (Sharafzadeh and Bahmani., 2014). *Thymus* genus constitutes around 350 species of aromatic herbs, perennials and shrubs which are found predominantly in the Mediterranean constituency, North Africa, Asia and Southern Europe (Maksimovic et al., 2008). Thymus species leaves and flowering parts have been comprehensively used as tonic, antitussive, antiseptic, carminative, herbal tea and for treating colds (Maksimovic et al., 2008; Rota et al., 2008). In food industries and academia, the chemical composition, antimicrobial and biological activities of the essential oil of *Thymus* and its extracts have recently been of immense interest (Hussain et al., 2010; Karaman et al., 2001; Rasooli and Mirmostafa, 2002; Rota et al., 2008; Verma et al., 2010). The present research work was therefore designed to investigate the phytochemical and antimicrobial potentials of less explored *T. linearis* against multidrug resistance (MDR) bacterial and important fungal strains.

Materials and Methods

Plant collection and extraction

Plant material was collected in August 2012 from peripheries of famous Lake Saif-ul-Malook, positioned in Naran valley and was identified by plant taxonomist

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Table I

Phytochemical investigation of *Thymus linearis*

Phytochemicals	Plant extracts Crude (methanol)	Plant extracts *n*-Hexane	Plant extracts Chloroform	Plant extracts Ethyl acetate	Plant extracts *n*-Butanol
Reducing sugar	Present	Absent	Absent	Absent	Absent
Anthraquinone	Absent	Absent	Absent	Absent	Absent
Terpenoids	Present	Absent	Present	Present	Present
Flavonoids	Present	Present	Absent	Present	Absent
Saponins	Absent	Absent	Absent	Absent	Absent
Tannins	Present	Present	Present	Absent	Present
Alkaloids	Present	Absent	Absent	Present	Absent
Glycosides	Present	Absent	Absent	Absent	Absent
Phlobatannins	Absent	Absent	Absent	Absent	Absent

Dr. M. Ibrar, Department of Botany, University of Peshawar. The plant was then shade dried and transformed into powder form by grinding. The powdered plant material was soaked in methanol for 15 days and was extracted with the same solvent three times at room temperature. The crude extract was then filtered through Whatman No. 1 filter paper. The filtered crude extract was fractionated with *n*-hexane, chloroform, ethyl acetate and butanol by standard procedures (Gracelin et al., 2012).

Phytochemical investigation

Qualitative tests were perfo-rmed on plant extracts by using standard protocols (Kayani et al., 2007; Ayoola et al., 2008) for recognition of flavonoids, carbohydrates, terpenoids, saponins, alkaloids, tannins etc.

Fourier transform infrared spectroscopy (FTIR)

The IR Pretige-21 FTIR model (Shimadzu, Japan) was used with IR solutions software. FTIR spectroscopy was performed for all the extracts in dried form by the method used by Ishaq et al (2014).

Collection and identification of bacterial and fungal cultures

Bacterial and fungal strains were collected from the microbiology laboratory of the Department of Microbiology, Abasyn University Peshawar. Nine bacterial strains (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus epidermidis, Citrobacter freundii, Providencia, Proteus vulgaris, Salmonella typhi)* and 4 fungal strains (*Penicillium, Rhizopus, Acromonium, Aspergillus)* were obtained and identified by their specific staining, biochemical and morphological characteristics.

Evaluation of multi-drug resistance pattern of the test bacterial strains

Prior to the assessment of antimicrobial potentials of extracts, MDR studies were conducted on all bacterial strains. The antimicrobial activity was measured through disc diffusion method using Muller Hinton agar. The sensitivity of 10 antibiotics was tested against the formerly mentioned 9 bacterial strains and the procedure was repeated three times. All the media plates were incubated for 24 hours at 37°C.

Assessment of antibacterial potentials of extracts

Antibacterial activity of *T. linearis* was executed by agar well diffusion method. In nutrient broth, bacterial strains were sub-cultured at 37°C for 24 hours. On sterile Muller Hinton agar plat, 100 µL of standard inoculums (0.5 MacFarland turbidity standards, 10^{-6} CFU/mL) of each test bacterial strain was spread. In triplicate wells of agar plates, 50 µL of each plant extract that is crude (TCRE), chloroform (TCHE), n-hexane (THXE), ethyl acetate (TEAE) and butanol (TBE) was poured and then incubated at 37°C for 24 hours. The zone of inhibition (ZI) was recorded for each plant extract to the nearest size in millimeters (mm).

Assessment of antifungal activity of extracts

Well diffusion method was also used for the evaluation of antifungal activity of *T. linearis*. On Sabouraud's Dextrose Agar plates (SDA), test fungal strains were sub-cultured for 3-5 days at 28°C. Wells were made through 6 mm diameter sterile cork borer in uncontaminated fresh SDA plates. Then fungal strains were spread on these plates. Each *T. linearis* extract (50 µL) was introduced into the wells in triplicate. SDA plates were incubated at 28°C for 72 hours. ZI was recorded in mm.

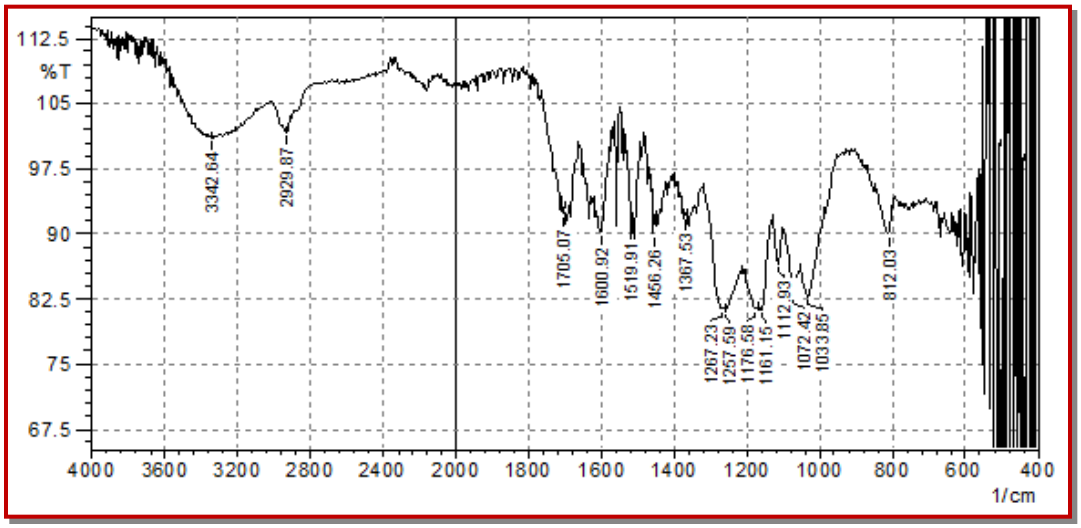

Figure 1: FTIR spectroscopy of methanol extract (crud) of *Thymus linearis*

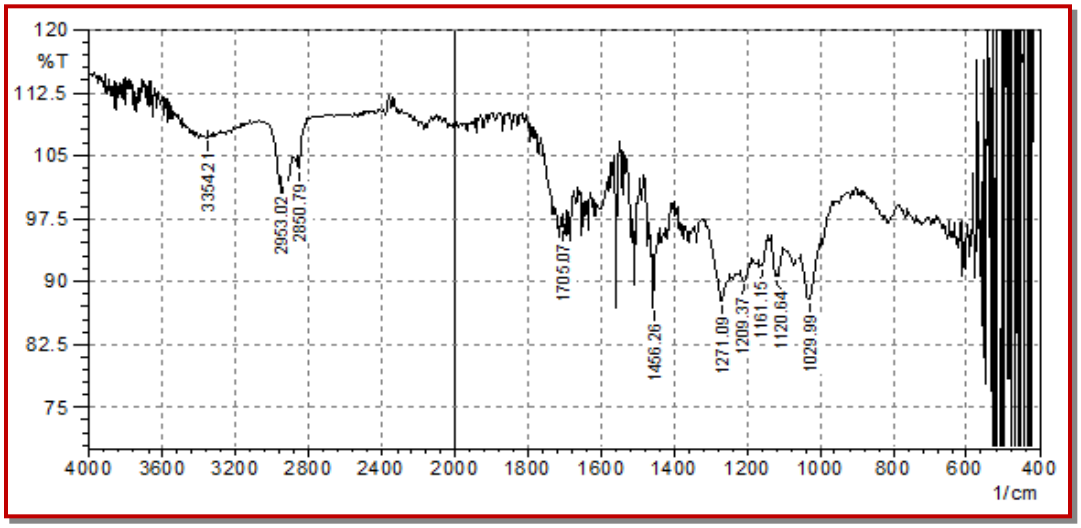

Figure 2: FTIR spectroscopy of chloroform extract of *Thymus linearis*

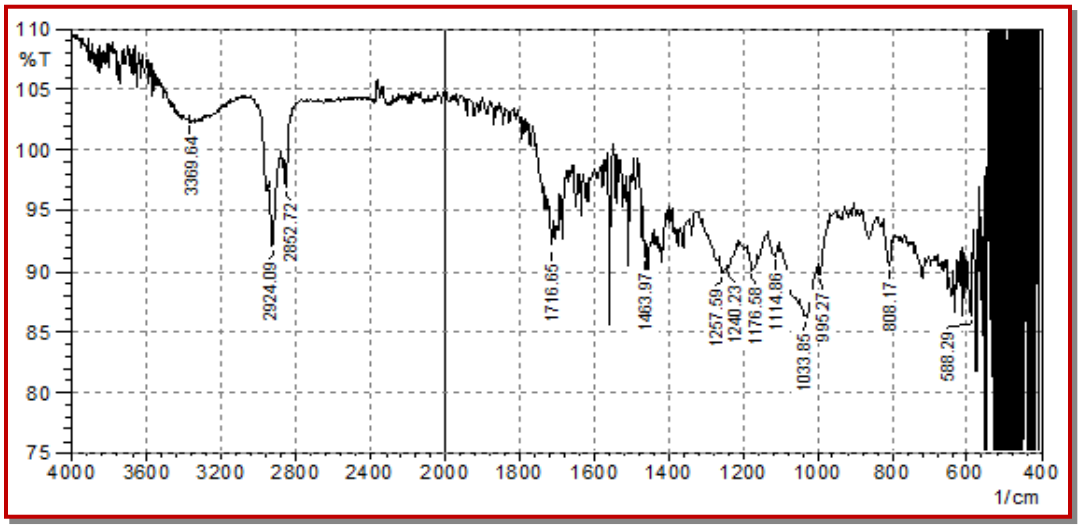

Figure 3: FTIR spectroscopy of *n*-hexane extract of *Thymus linearis*

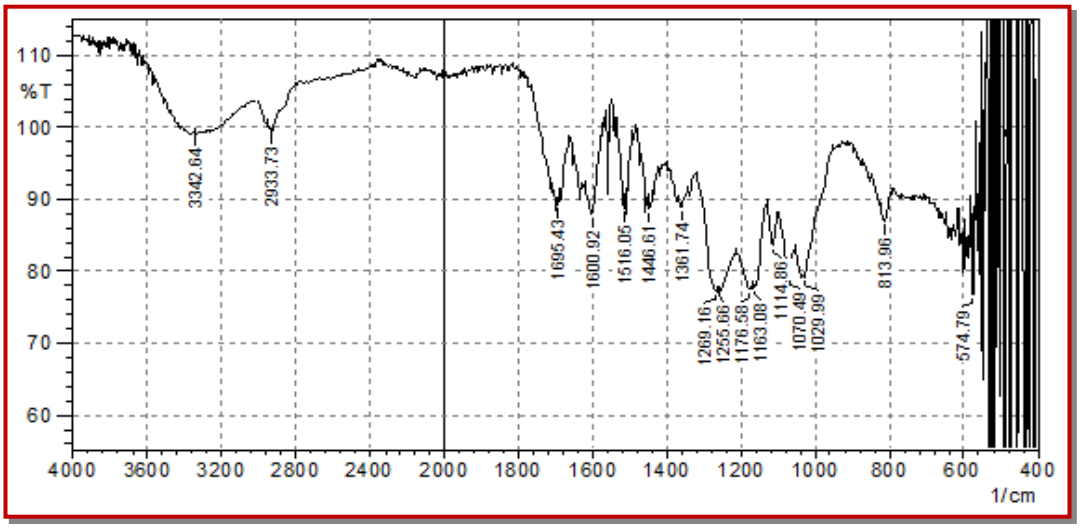

Figure 4: FTIR spectroscopy of ethyl acetate extract of *Thymus linearis*

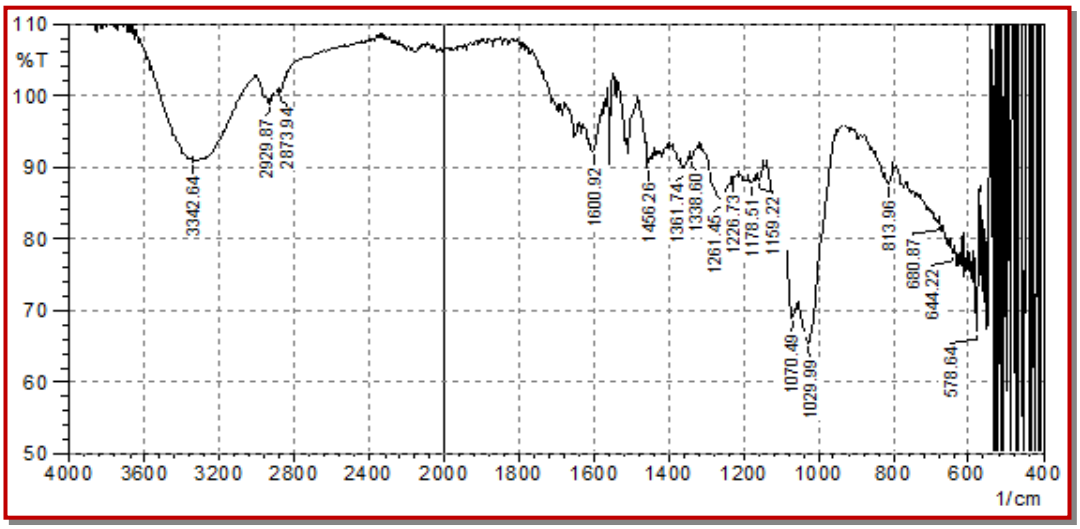

Figure 5: FTIR spectroscopy of butanol extract of *Thymus linearis*

Results

Phytochemical investigation of different extracts of *T. linearis revealed* the existence of terpenoids, flavonoids, tannins, glycosides, reducing sugars and alkaloids while the presence of anthraquinone, phlobatannins and saponins were not detected (Table I).

For compound identification of *T. linearis* extracts, FTIR spectroscopy was used. The infrared spectra of various extracts of the plant were recorded by IRPrestige-21 FTIR and execute under Infrared region between the ranges of 400-4000 cm^{-1}. Vibrational assignments, dominant peaks intensities and the wave number (cm^{-1}) were recorded from absorption spectra. The infrared prevailing peaks of plant extracts (Figures 1-5) recommend the presence of different compounds such as amides, aldehydes, carboxylic acids ethers, alcohols, ketones etc.

Ten common antibiotics were used to test the sensitivity of MDR bacterial strains. *Klebsiella pneumoniae* was found to be the most resistant bacterial strain exhibiting a diminutive sensitivity only against cefoperazone sulbactam (SCF) (17 mm) and levofloxacin (LEV) (4 mm) while showed resistance to the remaining 7 antibiotics. Similarly, *Pseudomonas aeruginosa* was only sensitive against ciprofloxacin (CIP) (30 mm), tetracycline (TE) (15 mm) and levofloxacin (LEV) (30 mm). *Staphylococcus epidermidis Provedensia* and *Proteus* were sensitive to 7 out of 10 antibiotics as evident from Table II.

In vitro antibacterial potentials of *T. linearis* indicates that TCRE has significant activities against *Salmonella typhi* (23 mm), *Staphylococcus aureus* (14 mm) and *Citrobacter freundii* (12 mm), while TCHE showed excellent activity (19 mm) against *Salmonella typhi* and THXE (14 mm) against *Escherichia coli*. Similarly, TBE has shown high ZI against *Klebsiella pneumoniae* (18 mm), *Providencia* (18 mm), *Staphylococcus aureus* (16 mm) and *Staphylococcus*

Table II

Drug resistance pattern of the test-bacterial strains

SL. No.	Microorganisms	Antibiotic disks with ZI (mm) representing sensitivity while (-) representing resistance									
		NA	CRO	SCF	CIP	SXT	CTX	AMP	CFP	TE	LEV
1	*E. coli*	-	-	12	15	-	-	-	5	2	14
2	*Staphylococcus aureus*	-	6	25	-	-	6	15	12	-	10
3	*Klebsiella pneumoniae*	-	-	17	-	-	-	-	-	-	4
4	*Pseudomonas aeruginosa*	-	-	-	30	-	-	-	-	15	30
5	*Staph. epidermitus*	20	-	30	28	-	30	20	30	-	30
6	*Citrobacter freundii*	-	14	15	-	-	-	19	9	4	-
7	*Providencia*	-	16	12	23	-	11	21	22	-	11
8	*Proteus vulgaris*	-	12	25	9	-	13	18	21	-	13
9	*Salmonella typhi*	-	12	28	-	-	12	-	27	-	11

NA= Naladixic acid, CRO= Ceftriaxone, SCF= Cefoperazone sulbactam, CIP= Ciprofloxacin, SXT= Trimethoprim/sulfamethoxazole, CTX= Cefotaxime, AMP= Ampicillin, CFP= Cefoperazone, TE= Tetracycline, LEV= Levofloxacin

Table III

Antibacterial and antifungal activities of *Thymus linearis* extracts

SL. No.	Microorganisms	ZI of TCRE				
1	*E. coli*	8	9	14	8	-
2	*Staphylococcus aureus*	14	11	-	10	16
3	*Klebsiella pneumoniae*	7	6	7	2	18
4	*Pseudomonas aeruginosa*	6	5	4	6	8
5	*Staph.epidermitus*	10	12	13	5	14
6	*Citrobacter freundii*	12	11	8	8	-
7	*Providencia*	11	10	10	9	18
8	*Proteus vulgaris*	9	9	12	3	13
9	*Salmonella typhi*	23	19	-	6	9
10	*Penicillium*	10	-	10	14	-
11	*Rhizopus*	14	-	4	10	5
12	*Acromonium*	6	-	3	-	-
13	*Aspergillus*	10	3	3	6	10

ZI (mm) representing sensitivity while (-) representing resistance

epidermidis (14 mm) (Table III).

Antifungal activities of different extracts of *T. linearis* are given in Table III. TCRE divulge fine activities against *Rhizopus* (14 mm), *Penicillium* and *Aspergillus fumigates* (10 mm each). Correspondingly, *n*-hexane extract was active against *Penicillium* (10 mm) while TEAE against *Penicillium* (14 mm) and *Rhizopus* (10 mm). TBE showed potential activity against *Aspergillus fumigates* (10 mm). TCHE were inactive against *Penicillium, Rhizopus* and *Acromonium.*

Discussion

The focus of scientist has been diverted to medicinally important plants due to growing emergence of antibiotic resistance. Along with antimicrobial potentials, valuable phytochemicals are also reported in plants. In search of new and more effective drugs, many plants have been studied globally for their phytochemical and antimicrobial actions. Consequently, this study has been designed to assess the phytochemical and antimicrobial potentials of methanol (crude), chloroform, n-hexane, ethyl acetate and butanol extracts of the whole plant of *T. linearis.*

Phytochemical analysis of *T. linearis* showed the existence of terpenoids, flavonoids, tannins, reducing sugars and alkaloids while anthraquinone, saponins and phlobatannins were not detected which is almost similar to the findings of other research work carried out in Pakistan on *T. linearis* (Alamgeer et al., 2014). FTIR investigation of our study results in the presence of various new compounds like amides, aldehydes, carboxylic acid, ethers, alcohol, ketones etc, the majority of which are not reported formerly to the best of our knowledge.

In the current study nine different bacterial strains were used, most of which were MDR to the test antibiotics . Our result manifest that among all used bacterial strains, *Klebsiella pneumoniae* was the most resistant strain (80% (8/10 antibiotics)) which is in line with 81.8% MDR investigated in 2013, locally (Hannan et al., 2013). On the contrary 70% (7/10 antibiotics) MDR *Pseudomonas aeruginosa* in our study is more than 50%

MDR in Peshawar Pakistan, recently reported (Ishaq et al., 2014). It was also evaluated that *Escherichia coli*, *Staphylococcus aureus, Proteus vulgaris, Citrobacter freundii* etc were rather of less MDR profile as compared to other studies (Hussain et al., 2014; Ishaq et al., 2014).

The *in vitro* antibacterial activity of *T. linearis* was assessed against MDR bacterial strains, most of which were human pathogens. Various extracts obtained from *T. linearis* exhibited good antibacterial activities against most of the MDR strains. For instance, *Staphylococcus aureus* was sensitive to TCRE, TCHE, TEAE and TBE, *Staphylococcus epidermitus* to TCRE, TCHE, THXE and TBE, and *Providencia* to TCRE, TCHE, THXE, TEAE and TBE. The results of our study are almost in accordance with antibacterial studies of a number of other *Thymus* species reported formerly (Karaman et al., 2001; Hussain et al., 2011; Rota et al., 2008; Maksimovic et al., 2008). It was also observed that most of the plant extracts were quite active in contrast to the used antibiotics against the various bacterial strains. Along with ample antibacterial potency *T. linearis* also has sufficient antifungal activities. TCRE and TEAE were found active against *Penillium* and *Rhizopus* while TCRE and TBE were potent against *Aspergillus*. *Acromonium* was almost non-responsive to the extracts. Our results are comparable with antifungal activities conducted on other *Thymus* species reported previously (Kucukbay et al., 2014; Karaman et al., 2001).

The current study confirms that extracts of *T. linearis* have considerable antibacterial and antifungal potentials along with important phytochemicals.

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