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Phytochemical analysis and antimicrobial study of *Fernandoa adenophylla* against multidrug resistant urinary tract infection pathogens

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Abstract

In the present study various solvent extracts from the leaves of *Fernandoa adenophylla* were screened for their phytochemical and antimicrobial potentials. Preliminary phytochemical screening of leaves extracts showed the presence of alkaloids, glycosides, flavonoids, terpenoids, tannins, steroids, reducing sugars and anthracenes. Fourier transform infrared spectroscopic studies showed the presence of different functional groups for compounds like alcohol, aldehydes and ketones, saturated and unsaturated hydrocarbons, amides, amines, carboxylic acids, esters, and ethers. All the extracts showed significant antibacterial activities when tested against eight multidrug resistant bacterial strains isolated from urinary tract infection patients. It was concluded that the leaves extracts of *F. adenophylla* have several vital phytochemical constituents and significant antibacterial activities against multidrug resistant bacterial strains causing urinary tract infections.

Introduction

The global emergence of multidrug resistant (MDR) bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failures (Hancock, 2005). One of the most common infectious diseases is urinary tract infection (UTI) (Sampson and Gravett, 1999) which has a high tendency of recurrence (Milan, 2006). Most of the studies have shown that UTI pathogens are resistant to the commonly prescribed antibiotics (Mussa-Aisien and Ibadin, 2003). Although many new antibiotics have been introduced, bacterial resistance is also continuously increasing (Hussain et al., 2014), which diverted the researchers towards the development of novel drugs from plant sources, having antimicrobial potentials (Maiyo et al., 2010).

Fernandoa adenophylla, an essential medicinal plant belongs to the family *Bignoniaceae* consisting of woody plants with approximately 860 species and 82 genera mainly growing in Africa, Central and South America (Lohmann, 2004). It is distributed in South and South-east Asian regions i.e. Burma, Pakistan, the Andaman Islands, East Bengal and Assam (Olmstead et al., 2009). The leaves and seeds of *F. adenophylla* are also used as an antimicrobial agent. It is used for the treatment of urinary tract infections as well as anti-diarrheal and anti -diabetic agents (Muhammad et al., 2012). As a folk medicine, it is employed for the treatment of amenorrhoea, night emission, premature ejaculation and skin diseases. It also has antifungal and antiseptic activities (Rahmatullah et al., 2010).

Therefore, the present research work has been designed to investigate the phytochemical constituents and antimicrobial activities of less investigated *F. adenophylla* against MDR bacterial strains causing UTIs.

Materials and Methods

Leaves collection, identification and extraction

Leaves were collected from the gardens of the University of Peshawar and identified by the Department of Botany, University of Peshawar. The leaves were shade dried and grinded to coarse powder (Sood and Sharma, 2010) using a crusher. Powdered leaves (150 g) were macerated in five different solvents (ethanol, methanol, ethyl acetate, n-hexane and purified water) (Cseke et al., 2006) for 24 hours at room temperature. The supernatant liquids were filtered and collected. The crude extracts were concentrated using rotary evaporator and the solvents were recollected. The concentrated extracts were dried at 60 ± 5°C using water bath. All the extracts were then preserved in separate labeled glass vials and stored at room temperature for further processes.

Phytochemical investigation of extracts

Qualitative tests were performed for each extract using standard protocols (Kayani et al., 2007; Ayoola et al., 2008) for the presence of different phytochemicals i.e. alkaloids, flavonoids, reducing sugars, terpenoids, saponins, tannins, steroids, glycoside and anthracene.

Fourier transform infrared spectroscopy

Fourier transformed infrared (FTIR) spectroscopy was carried out using IR Prestige-21 FTIR (Shimadzu, Japan) for the identification and confirmation of different compounds functional groups present in extracts in dried form from 400-4000 cm^{-1}.

Isolation and identification of bacterial strains

Urine samples from 150 UTI patients were collected from different tertiary care units of Peshawar, Pakistan. These samples were used for isolation of bacterial strains which were identified by specific morphological and biochemical characteristics in the laboratory of the Department of Microbiology, Abasyn University Peshawar. The pathogens isolated were: *Escherichia coli, Klebsiella pneumoniae, Citrobacter, Enterobacter, Pseudomonas aeruginosa, Acinetobacter, Providencia,* and *Methicillin-resistant Staphylococcus aureus* (MRSA).

Antimicrobial susceptibility testing for MDR UTI pathogens

Before evaluating the antimicrobial activity of plant extracts, all the isolated pathogenic microorganisms were tested for MDR profile. The antimicrobial susceptibility was carried out by disc diffusion method using Muller Hinton agar (MHA) as a medium (Ushimaru et al., 2007). The sensitivity of the isolated bacterial strains was tested against 10 commonly used antibiotics in triplicate. All the media plates were incubated for 24 hours at 37°C.

Antibacterial activity of plant extracts

Agar well diffusion method (Obeidat et al., 2012; Janovska et al., 2003) was applied for evaluation of antimicrobial activity of the leaves extracts of *F. adenophylla*. Each extract (10 mg) was dissolved in 1 mL of dimethyl sulfoxide to get a concentration of 10 mg/mL. On each sterile Muller Hinton agar plat, 100 µL of standard inoculum (0.5 MacFarland turbidity standards, 10^{-6} CFU/mL) of each test bacterial strain was spread with the help of sterilized cotton swabs while sterile borer was used for wells preparation. An amount of 100 µL of each extract i.e. ethanolic extract (EE), methanolic extract (ME), ethylacetate extract (EAE), *n*-hexane extract (nHE) and water extract (WE) was added through micropipette under aseptic conditions into specifically marked wells and then incubated at 37 ± 2° C for 18 hours. Zone of inhibition (ZI) was then measured to the closest point in millimeters (mm) (Bobbarala et al., 2009). The test was performed in triplicate and results were presented as mean ± standard deviation.

Results

Phytochemical screening

The phytochemical screening of *F. adenophylla* showed the presence of various essential constituents i.e. alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, reducing sugars and anthracene (Table I).

Compounds identification through FTIR spectroscopy

The peaks showed the presence of compounds like alcohol, amides, aldehyde, ketone, carboxylic acid, esters, ethers, amines, imides, amino acids, methyl substituted benzenes, alkyl azo compounds, cylclopentadienyls, organosulfonates and many other aliphatic, cyclic and aromatic compounds (Supplementary data, S1-S5).

Sensitivity of microorganisms

E. coli was found more sensitive to cefoperazone-sulbactam (24 mm), moderately sensitive to gentamicin (12 mm), while it was found resistant to ceftriaxone and moxifloxacin (8 mm), amoxycillin (9 mm), tetracycline, nalidixic acid, co-trimoxazole, ciprofloxacin and cefaclor (no ZI). *Klebsiella* showed more sensitivity to ceftriaxone and cefoperazone-sulbactam (28 mm each) which gradually decreases towards other antibiotics, i.e. cefaclor (23 mm), amoxycillin (20 mm), while it was found resistant towards moxifloxacin and gentamicin (10 mm each), tetracycline (09 mm), nalidixic acid, co-trimoxazole and ciprofloxacin (0 mm each). The *Citrobacter* was found more sensitive towards gentamicin (25 mm) and co-trimoxazole (24 mm) and showed gradual decrease in sensitivity towards cefoperazone-sulbactam (22 mm), ciprofloxacin and amoxycillin (20 mm each), moxifloxacin (18 mm) and cefaclor (15 mm) and showed less sensitivity towards tetracycline (12 mm), while it was resistant to ceftriaxone (12 mm) and nalidixic acid (no ZI).

Table I

Phytochemical screening of different extracts of *Fernandoa adenophylla*

Phytochemicals	Plant extracts				
	Ethanol	Methanol	Ethyl acetate	*n*-Hexane	Water
Alkaloids	+	+	+	+	+
Flavonoids	+	+	-	-	+
Tannins	+	+	-	-	+
Saponins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Glycosides	+	+	+	+	+
Reducing sugars	-	-	-	-	+
Anthracene	-	-	-	-	+

(+) = Present; (-) = Absent

Enterobacter was sensitive to ciprofloxacin (28 mm), gentamicin (24 mm) and cefoperazone-sulbactam (20 mm) and was found resistant to ceftriaxone (13 mm), amoxycillin and tetracycline (10 mm each), nalidixic acid, co-trimoxazole, and cefaclor (0 mm each) according to CLSI (2013) (Table II).

Pseudomonas showed the highest susceptibility profile towards cefoperazone-sulbactam (28 mm) which gradually decreases towards gentamicin (24 mm), ciprofloxacin (22 mm), ceftriaxone (20 mm), moxifloxacin (19 mm) and amoxycillin (18 mm). The organism showed moderate susceptibility to cefaclor (14 mm), while it was found resistant to co-trimoxazole (9 mm) and nalidixic acid (8 mm). *Acinetobacter* was observed to be more sensitive to co-trimoxazole (24 mm) and moxifloxacin (23 mm), cefaclor and gentamicin (22 mm each), amoxycillin (20 mm), tetracycline (19 mm) and cefoperazone-sulbactam (18 mm), and showed moderate sensitivity towards ciprofloxacin (19 mm) and ceftriaxone (15 mm). The organism was found resistant to nalidixic acid as no ZI was observed. *Providencia* was found less sensitive as compared to other organisms. Its maximum sensitivity decreases from cefoperazone-sulbactam (20 mm) towards amoxycillin (15 mm), ciprofloxacin (14 mm) and showed moderate sensitivity to moxifloxacin (12 mm). According to standard guidelines *Providencia* was found resistant to tetracycline (9 mm), ceftriaxone, nalidixic acid, co-trimoxazole, cefaclor and gentamicin (0 mm each). MRSA was more sensitive to gentamicin (20 mm) and cefoperazone-sulbactam (19 mm) and was found less sensitive to ciprofloxacin (16 mm) and amoxycillin and ceftriaxone (12 mm each), while it showed resistance to moxifloxacin (15 mm), tetracycline (14 mm), nalidixic acid (10 mm) and cefaclor (9 mm) and co-trimoxazole (no ZI) (Table II).

Antibacterial activity profile of the plant extracts

The leaves extracts *F. adenophylla* were tested against the MDR-UTI pathogens using cefoperazone-sulbactam

Table II

Culture sensitivity of bacterial strains

SL. No.	Microorganisms	Antibiotic discs with ZI (mm) against test organisms									
		TE	CRO	NA	SXT	AMC	MXF	CIP	CEC	CN	SCF
1	*E. coli*	R	8	R	R	9	8	R	R	12	24
2	*Klebsiella*	9	28	R	R	20	10	R	23	10	28
3	*Citrobacter*	12	12	R	24	20	18	20	15	25	22
4	*Enterobacter*	10	13	R	R	10	19	28	R	24	20
5	*Pseudomonas*	13	20	8	9	18	19	22	14	24	28
6	*Acinetobacter*	19	15	R	24	20	23	19	22	22	18
7	*Providencia*	9	R	R	R	15	12	14	R	R	20
8	MRSA	14	12	10	R	12	15	16	9	20	19

TE = Tetracycline CRO = Ceftriaxone, NA = Nalidixic acid, SXT = co-trimoxazole, AMC = Amoxycillin, MXF = Moxifloxacin, CIP = Ciprofloxacin, CEC = Cefaclor, CN = Gentamicin, SCF = Cefoperazone-Sulbactam, R = Resistant

as control. Antimicrobial activity of the extracts i.e. ethanol, methanol, ethyl acetate, n-hexane and water, was evaluated and their potency was quantitatively measured by the presence or absence of ZI (Table III).

The leaves extract of *F. adenophylla* showed significant antibacterial activity. All the test organisms were found sensitive to these extracts except *Klebsiella* which showed resistance to EE. The methanolic, ethanolic and water extracts have greater antibacterial activity than ethyl acetate and n-hexane extract.

The ethanol extract of *F. adenophylla* showed significant activity against all organisms except *Klebsiella*. The maximum ZI (27.33 mm) for EE was observed against *Enterobacter* and the lowest ZI (15.33 mm) was against *Providencia*. A ZI of 22.66 mm was observed against *Pseudomonas*, whereas the ZIs for *Acinetobacter*, *S. aureus*, *Citrobacter* and *Enterobacter* were 20.66 mm, 19.33 mm, 17.00 mm and 27.33 mm respectively. The ME showed 100% activity against all the strains. *Enterobacter* was found most sensitive with ZI of 34 mm while *Providencia* was found least sensitive (14.33 mm ZI). Activity of ME in terms of ZI against other species were, ZI of 17.33 mm for *E. coli*, 19.33 mm for *Pseudomonas*, 17.66 mm for *Klebsiella*, 19 mm for both *Acinetobacter* and MRSA and 17 mm for *Citrobacter*. Similarly, EAE also showed variable but significant activities against the tested microorganisms. *Enterobacter* was the most sensitive among all the strains with ZI of 26.66 mm, whereas *Klebsiella* was the least sensitive specie with ZI of 12.66 mm. Zone of inhibition for other strains were; 18.33 mm for *E. coli*, 18 mm for *Pseudomonas*, 15 mm for both *Acinetobacter* and *Citrobacter*, 17 mm for *Providencia* and 16.33 mm for MRSA. The nHE also showed maximum activity against *Enterobacter* with ZI of 28 mm while *Klebsiella* was the least sensitive with ZI of 14.66 mm. *E. coli* was found the second most sensitive organism with ZI of 18.33 mm while *Pseudomonas* (ZI of 16.33 mm), *MRSA* and *Citrobacter* (ZI of 16 mm each) exhibited almost similar sensitivity to the extract. Similarly *Acinetobacter* (ZI of 15.66 mm) and *Providencia* (ZI of 15.33 mm) showed approximately similar sensitivity profile to nHE. The WE was more active against *Enterobacter* with ZI of 23.33 mm followed by *MRSA* (20 mm ZI). Gradual decline in the sensitivity of microorganisms to WE was observed from *Acinetobacter* (ZI 19 mm), *Pseudomonas* (ZI 18.66 mm), *E. coli* (ZI 18 mm), *Citrobacter* (ZI 17.66 mm), *Providencia* (ZI 16.33 mm) to *Klebsiella* (ZI 16 mm) (Table III).

Discussion

The current results of phytochemical screening of all extracts of *F. adenophylla* have shown the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, anthracene and reducing sugars which are also reported previously (Kanchanapoom et al., 2001; Muhammad et al., 2012). Results obtained from the FTIR spectra of the extract have showed the presence of many compounds including aldehydes and ketones, amines, amides, imides, alcohols, carboxylic acids, esters and ethers, amino acids, methyl substituted benzenes, alkyl azo compounds, cylclopentadienyls, organosulfonates and many other aliphatic, cyclic and aromatic compounds, which are not reported in the previous studies to the best of our knowledge as no FTIR data has been found in the literature about *F. adenophylla*.

In the present study eight bacterial strains isolated from urine samples of UTI patients were used to test the antibacterial activities of plant extracts. The bacterial strains were found resistant to most of the antibiotics used in culture sensitivity study. According to standard guidelines (CLSI, 2013; NCCLS, 2012), the organisms were classified as MDR strains. The results showed that *E. coli* was found to be the most resistant bacteria i.e. to 90% antibiotics, *Enterobacter* to 70%, *Klebsiella* and *Providencia* to 60% and MRSA was found resistant to

Table III

Antibacterial activity profile of plant extracts

Organism	Ethanol	Methanol	Ethyl acetate	n-Hexane	Water	Control (SCF)
E. coli	20.33	17.33	18.33	18.33	18.00	22.00
Klebsiella	0.00	17.66	12.66	14.66	16.00	28.00
Citrobacter	17.00	17.00	15.00	16.00	17.66	26.00
Enterobacter	27.33	34.00	26.66	28.00	23.33	38.00
Pseudomonas	22.66	19.33	18.00	16.33	18.66	22.00
Acinetobacter	20.66	19.00	15.00	15.66	19.00	22.00
Providencia	15.33	14.33	17.00	15.33	16.33	24.00
MRSA	19.33	19.00	16.33	16.00	20.00	28.00

Extracts with ZI representing sensitivity or non-sensitivity in mm

50% antibiotics among all the tested bacterial strains. *Citrobacter* was resistant to 30% antibiotics, *Pseudomonas* to 20% and *Acinetobacter* was found resistant to only 10% antibiotics used in the study. The drug resistance pattern of some of these bacterial strains has also been reported by Ishaq et al. (2014) with little variation, from the same area i.e. Peshawar, Pakistan. In Ishaq et al. (2014) study, *E. coli* was found 78.6% resistant, *Pseudomonas* was 50%, *Klebsiella* and *Providencia* were 85.7%, *S. aureus* was 50% and *Citrobacter* was found 92.8% resistant to all the tested antibiotics. The variation found in the resistance profile of this study as compared to our study may be due to the nature of antibiotics used and sample.

All the leaves extracts of *F. adenophylla* were found to have good antibacterial activity. All the organisms under test were found sensitive to these extracts except *Klebsiella* which showed resistance to ethanolic extract. The ethanolic, methanolic and water extracts showed more antibacterial activity than n-hexane and ethyl acetate extracts. The EE showed maximum activity against *Enterobacter* (27.33 mm ZI) whereas it was found completely ineffective against *Klebsiella* (no ZI). The ME showed highest activity against *Enterobacter* (34 mm ZI) and lowest activity against *Providencia* (14.33 mm ZI). The EAE showed maximum activity against *Enterobacter* (26.66 mm ZI) while it was found least effective against *Klebsiella* (12.66 mm ZI). The nHE also showed optimum activity against *Enterobacter* (28 mm ZI) and showed minimum activity against *Klebsiella* (14.66 mm ZI). The WE showed maximum activity against *Enterobacter* (23.33 mm ZI) and minimum activity against *Klebsiella* (16 mm ZI). Most of the extracts were very effective against *Enterobacter* while least effective against *Klebsiella*. The previous work (Muhammad et al., 2012) on *F. adenophylla* (leaves and seeds) showed significant antimicrobial activity against different strains of bacteria including *B. subtilis*, *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*. According to Muhammad et al. (2012) study, extracts of *F. adenophylla* exhibited optimum activity against *S. aureus* and *E. coli* but showed no activity against *S. epidermidis*. In our study all the extracts showed optimum activity against these microorganisms which is in line with the study of Muhammad et al. (2012). Additionally, our study also covers the MDR bacterial strains in place of normal bacterial strains. There is no previous study on *F. adenophylla* extracts in which MDR strains have been tested. Furthermore, these MDR strains were isolated from the urine samples of UTI patients which give a new dimension to the present study.

In comparison to the antibiotics used in the culture sensitivity, the plants extracts were very active against all of the tested bacterial strains. In the present study, *E. coli* was resistant to 90% antibiotics but was found sensitive to EE, ME, EAE, nHE and WE. *Enterobacter* was found resistant to 70% antibiotics but showed sensitivities towards all extracts. Likewise, *Klebsiella* was found resistant to 60% antibiotics whereas it was found sensitive to ME, EE, nHE and WE. Similarly, *Providencia* and MRSA were resistant to 60% and 50% antibiotics respectively but found sensitive to all extracts. *Citrobacter, Pseudomonas* and *Acinetobacter* were found resistant to 30, 20 and 10% antibiotics respectively while they were found 100% sensitive to all the extracts.

Conclusion

Extracts of *F. adenophylla* had valuable phytochemical constituents along with significant antibacterial activity against MDR UTI pathogens. Different extracts have different antibacterial activities against MDR bacterial strains which showed the effectiveness of the plant extracts.

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Conflict of Interest

Authors declare no conflict of interest

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Supplementary Data

Table SI

IR spectra of ethanol extract for functional groups

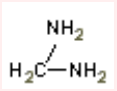
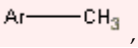
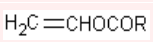
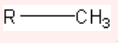
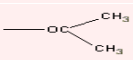
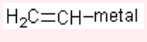
IR values (Frequency, cm^{-1})	Bond	Functional groups
3352	 , N–H stretch	1˚, 2˚ amines, amides Diamines
2922	 , C–H stretch	Methylbenzenes, Alkanes
2852	C–H stretch	Alkanes
1683		Vinyl ester
1456	 , C–H bend	Alkanes
1278	C–N stretch	Aromatic amines
1161	C–N stretch	Aliphatic amines
1033		Esters
997	 , == C–H bend	Cyclopentadienyls, Alkenes
661	–C=C–H: C–H bend	Alkynes

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Table SII

IR spectra of methanol extract for functional groups

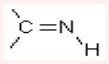
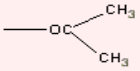
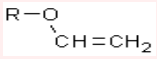
R values (Frequency, cm^{-1})	Bond	Functional groups
3329	 N–H stretch,	1˚, 2˚ amines, amides, Imines
2922	Ar--CH_3, C—H stretch	Aromatic compounds
2850	C–H stretch	Alkanes
2362	-C-N–H stretch	Amino acids
1687	C=O stretch	α-β–unsaturated aldehydes, Ketones
1456	C–H bend	Alkanes
1273	C–N stretch	aromatic amines
1255	C–N stretch	aromatic amines
1161	C–N stretch	aliphatic amines
1033		Esters
997	$H_2C{=}CH{-}metal$, =C–H bend	Cyclopentadienyls, Alkenes
813		Vinyl ether
667	–C=C–H, C–H bend	Alkynes
594	$-C{\equiv}CH$, C—Br	mono subst. alkynes, Alkyl halides

Table SIII

IR spectra of ethyl acetate extract for functional groups

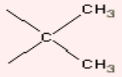
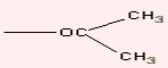
IR values (Frequency, cm^{-1})	Bond	Functional groups
2922	$Ar{-}CH_3$, C–H stretch	Methylbenzenes, Alkanes
2850	C--H stretch	Alkanes
1687	$H_2C{=}CHOCOR$	Vinyl ester
1456	$R{-}CH_3$, C–H bend	Alkanes
1373		Branched Alkanes
1317	C–O stretch	carboxylic acids, alcohols, ethers, esters
1274	C–N stretch	Aromatic amine
1165	C–N stretch	Aliphatic amine
1029		Esters
997	$H_2C{=}CH{-}metal$, =C–H bend	Cyclopentadienyls, Alkenes
661	–C=C–H: C–H bend	Alkynes

Table SIV

IR spectra of n-hexane extract for functional groups

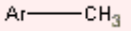
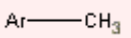
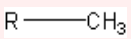
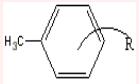
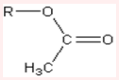
IR values (Frequency, cm^{-1})	Bond	Functional groups
3325	N–H stretch, >C=N-H	1˚, 2˚ amines, amides, Imines
2953	 , C–H stretch	Methylbenzenes, Alkanes
2916	 , C–H stretch	Methylbenzenes, Alkanes
2848	Ar-O-CH_3	Aromatic ethers
1732	C=O stretch	Aldehydes, saturated aliphatic
1712	C=O stretch	Ketones, saturated aliphatic
1639	–C=C– stretch	Alkenes
1456	 , C–H bend	Alkanes
1377	-C-C-	Branched Alkanes
1317	C–O stretch	Alcohols, carboxylic acids, ethers, esters
1242	-C-H Bend	Cycloalkanes
1163	C–N stretch	Aliphatic amines
1033	-O-C-CH_3	Esters
1026	-C-C-H	Cyclohexane
968	=C–H bend	Alkenes
777	R-CH=CH-R	Halogen substituted vinylene
719	C–H bend	Alkanes

Table SV

IR spectra of water extract for functional groups

IR values (Frequency, cm^{-1})	Bond	Functional groups
3236	O–H stretch, H–bonded	Alcohols, phenols
1583	N–H bend	1˚ amines
1558	C–C stretch (in–ring)	Aromatics, Alkyl azo compounds
1404	CH_3-C=O	Acyclic Ketones
1398	C=O stretch	Aldehydes, saturated aliphatic
1361	C–H rock	Alkanes
1311	C–O stretch	Alcohols, carboxylic acids, esters, ethers
1280	C–H wag (–CH2X)	Alkyl halides
1263	C–H wag (–CH2X)	Alkyl halides
1120	C–N stretch	Aliphatic amines
1093		Sat. sec Alcohol
1072	-CH_2-OH	Primary alcohol
1043		Methyl benzenes
871	C-O-SO_3	Organosulfate
657	–C≡C–H: C–H bend	Alkynes
607		Esters
597	C–Br stretch	Alkyl halides