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Research Article

Characterization of Metabolites Produced by *E. Coli* and Analysis of Its Chemical Compounds Using GC-MS

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ABSTRACT

Bioactives chemical compounds often referred to as secondary metabolites were analyzed using gas chromatography-mass spectroscopy (GC-MS) techniques, then the in vitro antibacterial and antifungal activity of the methanolic extract was evaluated. Twenty three bioactive compounds were identified in the methanolic extract of *E. coli*. GC-MS analysis of *E. coli* revealed the existence of the Dodecanoic acid ,3-hydroxy, 13-Tetradecynoic acid , methyl ester, Octahydrochromen-2-one , Octahydrochromen-2-one , 12,15-Octadecadiynoic acid methyl ester, 9-Tetradecen-1-ol , acetate , (E) , Propanamine , 3-(methylthio) , H-Pyrrole ,1-pentyl , N-[3-[N Aziridyl]propylidene tetrahydrofurfurylamine, Cyclopentadecanone , Benzeneethanamine , 5H-Pyrindine , dl-Allo-cystathionine , Adenosine ,4'-methylaminoformyl -4'-deshydroxymethyl-N- , Uric acid , Hexadecanol , 2-methyl, Spiculesporic acid , N,N' Bis(Carbobenzyloxy) lysine methyl (ester) , Methoxyphenoxyformamide N-methyl-N-[4-(1-pyrrolidinyl)-2 , Oxime -,methoxy-phenyl , Acetamide , N-methyl-N-[4-[4- fluoro-1- hexahydropyridyl]-2-buty , Cyclohexane , 1R-acetamido-2,3-cis-epoxy-4-trans-acetoxy , N-t Butyl-N'-[1,1-dimethyl-2 , thiosulfatoethyl]-1,3- , Actinomycin C2. *Cassia angustifolia* (Crude) was very highly active (7.05±0.26) mm. *E. coli* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *E. coli* can be useful.

Keywords: Antifungal activity, *E. coli*, GC-MS, Secondary metabolites.

INTRODUCTION

Escherichia coli is a rod-shaped, Gram-negative facultative anaerobe. Most *E. coli* strains are nonpathogenic. Although *E. coli* lives in the human gut as a commensal organism, certain pathogenic strains can exit the human gut and cause disease at other anatomical sites[1,2]. One example of this phenomenon is uropathogenic *E. coli* (UPEC), which causes 70% of UTIs[1]. UPEC strains are thought to originate in the patient's fecal flora and be spread via fecal contamination to the periurethral area. The bacteria can then ascend the urethra into the bladder and cause a UTI. UPEC strains express a variety of virulence factors including adhesive appendages, known as pili, and toxins that allow them to infect the bladder. *Escherichia coli* presents a large array of genetic subtypes defined by the somatic (O) and flagellar antigen (H). Most subtypes are harmless whilst some can cause severe diarrhoea[1]. The most common bacterial uropathogens in UTI are: Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis and Enterobacter cloacae[2,3]. P. mirabilis is a common cause of UTI in the complicated urinary tract, most frequently in patients with indwelling catheters or structural abnormalities of the urinary tract[4,5]. P. mirabilis expresses several virulence factor involved in uropathogenesis like adhesins, flagella, toxins, quorum-sensing, enzymes and immune invasion[6-8]. The aims of this research were analysis of the bioactive chemical products and evaluation of antibacterial activity.

MATERIALS AND METHODS

Growth conditions and determination of metabolites

E. coli strain was isolated from bronchitis patients and obtained from Maternity and children hospital. Subcultures were obtained on the nutrient agar for 48 hrs. at 22°C. The mixture was incubated at 4ºC for 10 min and then shook for 10 min at 130 rpm. Metabolites was separated from the liquid culture and evaporated to dryness with a rotary evaporator at 45ºC. The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4ºC for 24 h before being used for GC-MS[9-21]. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library as well as on comparison of their retention indices either with those of authentic compounds or with literature values[22-29].

Spectral analysis of bioactive chemical compounds using gas chromatography-mass spectrometry (GC/MS)

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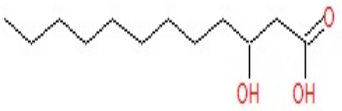
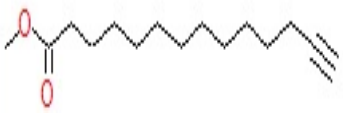
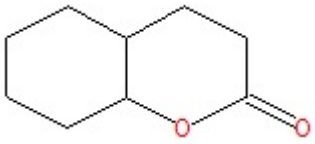
Table 1: Bioactive chemical compounds identified in methanolic extract of *E. coli*.



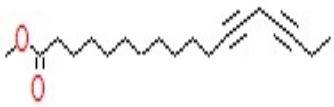
Dodecanoic acid ,3-hydroxy
RT= 3.144
Mw= 216.1725445
Pharmacological activity:
Anti-apoptotic and anti-inflammatory effects



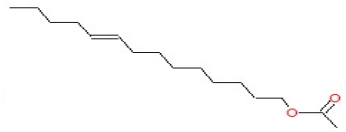
13-Tetradecynoic acid, methyl ester
RT= 3.218
Mw= 238.19328
Pharmacological activity:
anti-inflammatory, and antitoxin effects



Octahydrochromen-2-one
RT= 3.315
Mw= 154.09938
Pharmacological activity:
Unkown

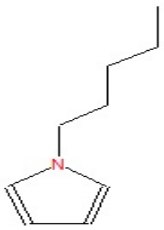


12,15-Octadecadiynoic acid, methyl ester
RT= 3.510
Mw= 290.22458
Pharmacological activity:
antioxidant, anti-inflammatory, antimicrobial

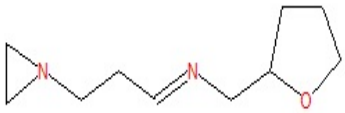


9-Tetradecen-1-ol, acetate, (E)
RT= 3.865
Mw= 254.22458
Pharmacological activity:
anti-inflammatory and hepatoprotective *properties*

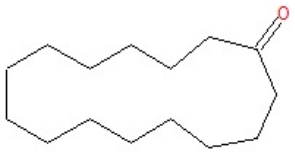
1-Propanamine, 3-(methylthio)
RT= 3.945
Mw= 105.06122
Pharmacological activity:
Anti-Inflammatory And Analgesic Agents



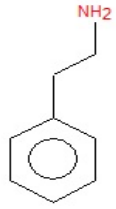
1H-Pyrrole ,1-pentyl
RT= 4.425
Mw= 137.120449
Pharmacological activity:
antidepressant



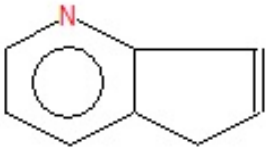
N-[3-[N-Aziridyl]propylidene] tetrahydrofurfurylamine
RT= 4.603
Mw= 182.141913
Pharmacological activity:
anti-diabetic, anti-inflammatory, antioxidant



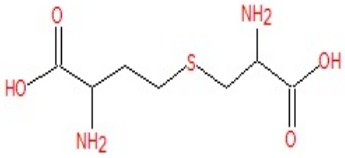
Cyclopentadecanone
RT= 4.620
Mw= 224.214016
Pharmacological activity:
anti-microbial activity, antioxidant and antiinflammatory activity



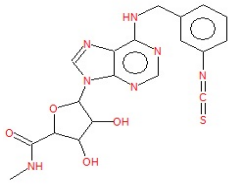
Benzeneethanamine
RT= 5.244
Mw=121.0891495
Pharmacological activity:antioxidant, antimicrobial, anti- inflammatory



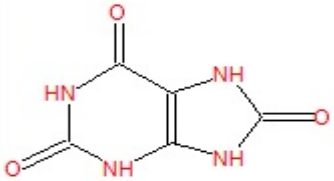
5H-Pyrindine
RT= 7.395
Mw= 117.0578494
Pharmacological activity:
Anti-inflammatory and analgesic activities



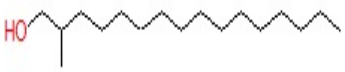
dl-Allo-cystathionine
RT= 8.213
Mw= 222.067428
Pharmacological activity:
anti- inflammatory



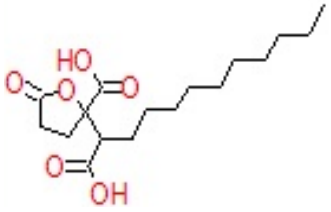
Adenosine, 4'-methylaminoformyl -4'-deshydroxymethyl-N- RT= 9.867
Mw=441.121923
Pharmacological activity:
anti-inflammatory



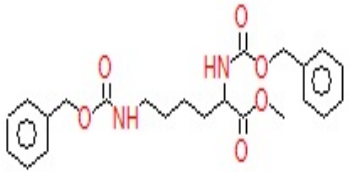
Uric acid
RT= 12.963
Mw=168.02834
Pharmacological activity:



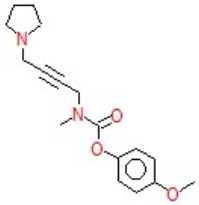
1-Hexadecanol, 2-methyl
RT= 14.387
Mw=256.276615
Pharmacological activity:
Anti-bacterial activity



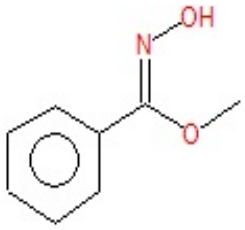
Spiculesporic acid
RT= 16.665
Mw=328.18859
Pharmacological activity:
Unknown



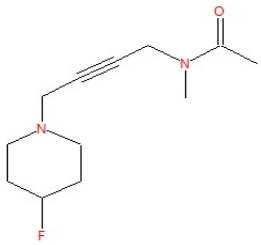
N, N'-Bis(Carbobenzyloxy) -lysine methyl (ester)
RT= 16.831
Mw=428.194736
Pharmacological activity:
anti-cancer activity



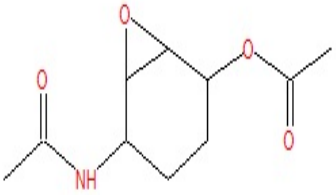
4-Methoxyphenoxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2
RT= 17.706
Mw=302.163042
Pharmacological activity:
anti-inflammatory activities



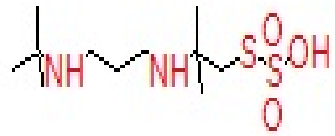
Oxime -,methoxy-phenyl
RT= 3.384
Mw= 151.063329
Pharmacological activity:
antimicrobial activity



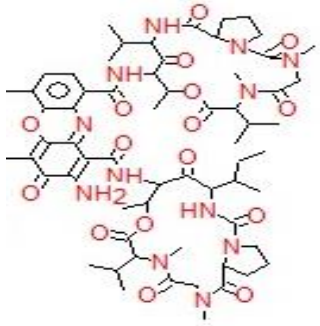
Acetamide , N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-buty
RT= 4.214
Mw= 226.148142
Pharmacological activity:
Unkown



Cyclohexane , 1R-acetamido-2,3-cis-epoxy-4-trans-acetoxy
RT= 5.124
Mw= 213.100108
Pharmacological activity:
Unkown



N-t-Butyl-N'-[1,1-dimethyl-2 - thiosulfatoethyl]-1,3-propanedia
RT= 9.352
Mw= 298.138485
Pharmacological activity:
Unknown



Actinomycin C2
RT= 15.681
Mw=1268.64413
Pharmacological activity:
Anti-Infective Agents

Analysis was conducted using GC-MS (Agilent 789 A) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250 Cº). Ionization voltage was 70 eV and ion source temperature was 230oC. Scan range was 41- 450 amu. The components were identified by comparing their retention times to those of authentic samples of WILEY MASS SPECTRAL DATA BASE Library[30-37].

Determination of antibacterial activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Piper nigrum*, *Zingiber officinale*, *Gramineae poaceae*, *Nerium olender*, *Ricinus communis*, *Datura stramonium*, *Linum usitatissimum*, *Anastatica hierochuntica*, *Cassia angustifolia*, *Euphorbia lathyrus*, *Rosmarinus oficinalis*, *Mentha viridis*, *Artemisia annua*, *Quercus infectoria*, *Citrullus colocynthis*, *Althaea rosea*, *Coriandrum sativum*, *Origanum vulgare*, *Urtica dioica*, *Equisetum arvense*, *Foeniculum vulgare*, *Nigella sativa*, *Ocimum basilicum*, *Punica granatum*, *Punica granatum* and *Cinnamomum zeylanicum*) were delivered into the wells[38-45]. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by SPSS software) Version 9.1.

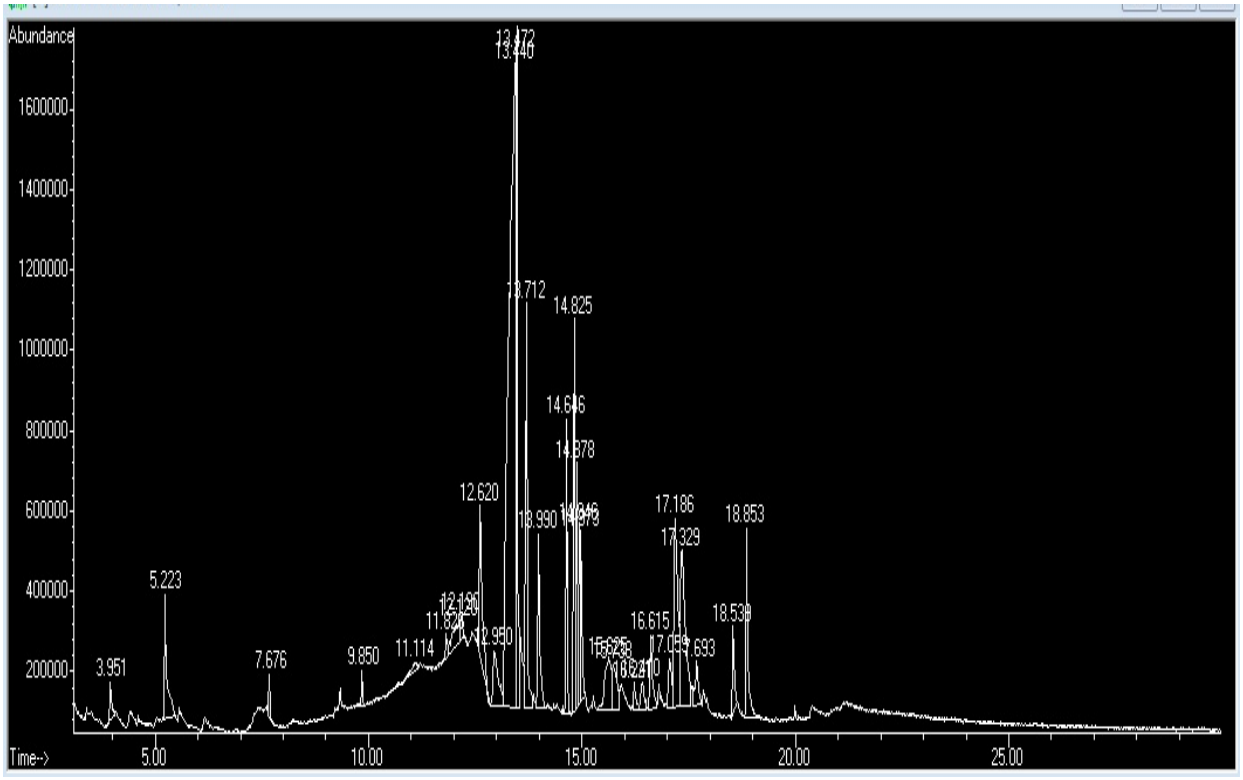


Figure 1: GC-MS chromatogram of methanolic extract of *E. coli*.

Table 2: Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of medicinal plants to *E. coli*.

S. No.	Plant	Zone of inhibition (mm)
1.	*Piper nigrum* (Crude)	5.99±0.21
2.	*Zingiber officinale* (Crude)	4.89±0.21
3.	*Gramineae poaceae* (Crude)	6.98±0.24
4.	*Nerium olender* (Alkaloids)	4.47±0.21
5.	*Ricinus communis* (Alkaloids)	3.00±0.20
6.	*Datura stramonium*(Alkaloids)	4.01±0.21
7.	*Linum usitatissimum* (Crude)	5.00±0.25
8.	*Anastatica hierochuntica* (Crude)	4.87±0.19
9.	*Linum usitatissimum* (Crude)	4.00±0.20
10.	*Cassia angustifolia* (Crude)	7.05±0.26
11.	*Euphorbia lathyrus* (Crude)	6.07±0.28
12.	*Rosmarinus oficinalis* (Crude)	4.70±0.20
13.	*Mentha viridis* (Crude)	6.00±0.25
14.	*Artemisia annua* (Crude)	4.99±0.20
15.	*Quercus infectoria* (Crude)	7.00±0.21
16.	*Citrullus colocynthis* (Crude)	5.21±0.21
17.	*Althaea rosea* (Crude)	4.99±0.20
18.	*Coriandrum sativum* (Crude)	5.00±0.21
19.	*Melia azedarach* (Crude)	4.68±0.21
20.	*Origanum vulgare* (Crude)	6.05±0.24
21.	*Urtica dioica* (Crude)	3.08±0.15
22.	*Equisetum arvense* (Crude)	5.89±0.14
23.	*Foeniculum vulgare* (Crude)	3.62±0.19
24.	*Nigella sativa* (Crude)	4.00±0.20
25.	*Ocimum basilicum* (Crude)	3.99±0.19
30.	Control	0.00

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *E. coli*, shown in Table 1. The GC-MS chromatogram of the twenty three peaks of the compounds detected was shown in Figure 1. The First set up peak were determined to be Dodecanoic acid, 3-hydroxy. The second peak indicated to be 13-Tetradecynoic acid, methyl ester. The next peaks considered to be Octahydrochromen-2-one, Octahydrochromen-2-one, 12,15-Octadecadiynoic acid methyl ester, 9-Tetradecen-1-ol, acetate, (E), Propanamine, 3-(methylthio), H-Pyrrole,1-pentyl, N-[3-[N Aziridyl]propylidene tetrahydrofurfurylamine, Cyclopentadecanone, Benzeneethanamine, 5H-Pyrindine, dl-Allo-cystathionine, Adenosine, 4'-methylaminoformyl - 4'-deshydroxymethyl-N-, Uric acid, Hexadecanol, 2-methyl, Spiculesporic acid, N,N' Bis(Carbobenzyloxy) lysine methyl (ester), Methoxyphenoxyformamide N-methyl-N-[4-(1-pyrrolidinyl)-2, Oxime -,methoxy-phenyl, Acetamide, N-methyl-N-[4-[4- fluoro-1-hexahydropyridyl]-2-buty, Cyclohexane, 1R-acetamido-2,3-cis-epoxy-4-trans-acetoxy, N-t Butyl-N'-[1,1-dimethyl-2, thiosulfatoethyl]-1,3-, Actinomycin C2. In agar well diffusion method the selected medicinal plants (*Piper nigrum*, *Zingiber officinale*, *Gramineae poaceae*, *Nerium olender*, *Ricinus communis*, *Datura stramonium*, *Linum usitatissimum*, *Anastatica hierochuntica*, *Cassia angustifolia*, *Euphorbia lathyrus*, *Rosmarinus oficinalis*, *Mentha viridis*, *Artemisia annua*, *Quercus infectoria*, *Citrullus colocynthis*, *Althaea rosea*, *Coriandrum sativum*, *Origanum vulgare*, *Urtica dioica*, *Equisetum arvense*, *Foeniculum vulgare*, *Nigella sativa*, *Ocimum basilicum*,

Punica granatum, *Punica granatum* and *Cinnamomum zeylanicum*) were effective against *Aspergillus terreus*, Table 2. *Cassia angustifolia* (Crude) was very highly active (7.05±0.26) mm against *E. coli*. *E. coli* O157:H7 is commonly found in the intestines of cattle and cross contamination of other parts of the animal is possible when slaughtering is not properly done. The bacterium is also found naturally in the intestines of other animals like pigs, sheep, goats and deer[46-50]. Natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for development of new lead chemicals for pharmaceutical companies. Gastrointestinal infections with enterotoxigenic Escherichia coli (ETEC) pose a major health problem among children younger than five years old in developing countries[51]. Over the last 20 years, numerous studies have been conducted on plants (using water and organic solvent extractions) including essential oils of what is known as medicinal plants. Although traditional medicine have associated certain plant products with healing of disease including gastrointestinal disorders, the mechanism accounting for antibacterial activities against enterobacteria which some are responsible for causing diarrhea are not thoroughly understood[52]. In vitro tests (disk diffusion and broth dilution methods) were conducted in search for effective treatment of diarrheal disease causing bacteria. In addition, plants with significant activity against enteropathogens could offer alternative methods to treat drug resistant enteric infections[53].

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