Tromethamine and Dodecanol-Like Compounds Appear to be the Major Secondary Metabolites of *Streptomyces Decoyicus* (M*)

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Research Article

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Abstract

An isolate of *Streptomyces decoyicus* was identified by the sequencing of 16S rRNA gene. It was grown on solid media (250 ml) and secondary metabolites were extracted with *n*-butanol (100 ml). The extract was dried and run in a sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE, 10%). Two main bands obtained were sliced and the metabolites were regained in *n*-butanol (100µl). These two samples were then identified by gas-chromatography-mass spectrometry (GC-MS), and Fourier-transform infrared spectroscopy (FT-IR). The results demonstrated that tromethamine- and 1-dodecanol were the main constituents (band 1: 61% and 17.7%; band 2: 41% and 54%, respectively). This finding maintained that the isolate of *Streptomyces decoyicus* produced high amounts tromethamine- and 1-dodecanol-like compounds under the conditions investigated.

Introduction

The genus *Streptomyces* includes gram-positive bacteria with branching hyphae (Embley and Stackebrandt, 1994; González et al., 2005) that can differentiate into chains of reproductive spores (Madigan et al., 2000). It has a great industrial importance owing to its capacity to produce diverse secondary metabolites that make up 80% of the industrially availble antibiotics (Tanaka and Omura 1990; Woodruff 1999; Takahashi and Omura 2003). The ability of *Streptomyces* to produce bioactive products is not a fixed trait (Bull et al. 2000; Watve et al. 2001; Hayakawa et al., 2004) and under defined stress conditions, the types and amounts the secondary metabolites can be modulated (Reddy et al., 2011; da Silva et al., 2012; Al-Hulu, 2013; Khattab et al., 2016; Waksman, 1961; Bundale et al., 2015).

One-dodecanol or lauryl alcohol ($C_{12}H_2O_6$) is a colourless fatty substance in solution. It has an unpleasant odour at high concentrations, although emitting a pleasant floral scent at low concentrations (Mureşan et al., 2014; Saini, 2016). It is a natural compound in apples (Mureşan et al. 2014) and coriander (Saini, 2016). It can be obtained as a volatile by-product in blue crab processing and it is mostly produced from palm kernel or coconut oil (Chung and Cadwallader, 1993). In pure form, it appears to be a highly versatile product. It is used in lubricants and monolithic polymers, detergents and soaps, shampoo, body wash- and shaving gels as well as hair dyes. Though less frequently, it is also used as emulsifying and wetting agents. It has been attested to be safe in food products (Motteran et al., 2019; Watanabe et al., 2005).

Trometamol, also known as THAM, 2-amino-2-(hydroxymethyl)-1,3-propanediol or tris base, tris(hydroxymethyl)aminomethane, belongs to the class of organic compounds known as 1,2-aminoalcohols. These are organic compounds containing an alkyl chain with an amine group attached to the C1 atom and an alcohol group attached to the C2 atom. Tromethamine is a weak organic acid and it can slow acid-catalyzed hydrolyses. Thus, it is used as a drug for the prevention and correction of metabolic acidosis. Tromethamine is also a parent compound for other conversion products, including bis-tris, bis-tris propane, and N-tris(hydroxymethyl)methylglycine (Dijkmans et al., 2017).

In this study, an isolate of *Streptomyces decoyicus* was also used to produce tromethamine- and 1-dodecanol-like compounds. These two metabolites were again the predominant secondary metabolites that could be resolved in an SDS-polyacrylamide gel and separated into two distinct bands. Elution from the gel was again achieved by using *n*-butanol. After the elution the samples were subsequently identified by mass spectrometry (GC-MS) and confirmed by Fourier-transform infrared spectroscopy (FT-IR).

Materials And Methods

Collection of soil samples

Streptomyces isolates were obtained from red soil samples (Çamlıbel, Tokat, Turkey).

Isolate purification

Soil sample, 10g, was homogenized in 90 ml of NaCl (0.85%) for 2h at room temperature. Dilutions, up to 10^{-5}, were spread, to obtain single colonies, on LB-agar plates (g/L-1 10g peptone, 5g yeast extract, 10g NaCl, and 15g agar) and incubation took place at 37ºC for 48h. Morphological characterisation involved Gram staining and light microscope. Pure isolates were then stored in 20% glycerol at -80°C. (Usha Nandhini et al. 2018).

Species identification by 16S rRNA gene sequencing

Genomic DNA was prepared by utilizing the HotSHOT DNA extraction method (Lunt, 2017). The V3-V4 variable region of 16S rRNA were amplified using the primers from Klindworth et al. (2013). DNA sequencing was performed in an Illumina MiSeq instrument in paired-end mode with 2x 250 nucleotide read length at Sivas Cumhuriyet University Advanced Technology Research and Application Center (CUTAM). Sequencing data were queried in the BLAST database and the results were recorded Camacho et al., 2009). Isolates belonged to *Streptomyces decoyicus* were multiple aligned using MAFFT program (Katoh et al., 2009). In addition, phylogenetic trees were created by using the Kimura-2 genetic distance model and Neighbour-joining (NG) method to determine the relationship between the samples using aligned data Kimura, 1980; Tamuraet al., 2013). Bootstrap method was used to test the tree topology and it was repeated for 500 times. An accession number for these sequences has also been obtained from GenBank: MZ159946.

Secondary metabolite production

Streptomyces decoyicus cells in glycerol stocks were first activated by growing overnight at 37°C in 50 ml LB. Secondary metabolites were produced in solid medium (250 ml of LB with 0.15% agar in 25 cm diameter glass pots) by spreading 100µl of the overnight culture and by incubation for 10d or until the colour of the agar darkened. The metabolites were then eluted into 100 ml of n-butanol overnight at room temperature. The extract was clarified by filtration and the organic solvent was evaporated at 70°C. The dry extract was stored at -20 °C (Fleck et al. 1972; Çetinkaya, 2021; Çetinkaya et al. 2021).

Thin layer chromatography

A portion of the total extract was resolved by thin layer chromatography (silica gel 60, Merck) using chloroform: methanol (10:1, v/v). Bands were visualized under ultraviolet light. Retention factors (Rf) of the bands were then calculated (Bundale et al., 2018).

Purification of secondary metabolites

The secondary metabolite extract was resolved in 10% master gel [30% acrylamide: bisacrylamide, 1.5M Tris-HCl, pH 8.8, 10% SDS, 10% ammonium persulfate (APS)]. A stacking gel (5%) was loaded onto the main gel (30% acrylamide: bisacrylamide, 1.5M Tris-HCl, pH 6.8; 10% SDS, 10% APS). Electrophoresis was performed for 2h at 70V. Bands visible to the naked eye were sliced out and metabolites were separated in *n*-butanol overnight at 4 °C.

Identification of secondary metabolite contents

The samples eluted were analysed by gas chromatography-mass spectrometry (GC-MS, Shimadzu, Model: GCMS –QP 2010 ULTRA, Research Centre Laboratories, Kastamonu University) and Fourier Transform Infrared Spectroscopy (FT-IR, Bruker, Tensor II).

Results

Identification of the Streptomyces isolate

16S rRNA gene was sequenced and the sequence data was stored in GenBank (accession number: MZ159946). Homology search identified the isolate to be *Streptomyces decoyicus* (Fig. 1). This organism optimally grew at pH7 at 37°C. It formed yellowish colonies on agar medium. Gram staining was used for morphological identification under the light microscope.

Resolution of the crude extract

Crude *n*-butanol extract was first run in thin layer chromatography sheets and two visible bands were obtained. The Rf values of these bands were 0.30 and the 0.23 (Fig. 2). These wo bands were also shown in SDS-PAGE gels (Fig. 3). The bands were cut out from the gel and their contents were eluted overnight in *n*-butanol. Dry extracts were obtained by evaporating the organic solvent and were then analysed by FT-IR and GC-MS.

Metabolite identification and characterization

The elution samples were identified by GC-MS analysis (Fig. 4). One of the bands, M1, contained 61.12% tromethamine, 17.77% 1-dodecanol, 7.71% 13-docosenamide (Table 1). The band M2 had 54.06% 1-dodecanol and 41.16% tromethamine (Table 2). The presence of these compounds was also verified by FTIR (Fig. 5): v max. (cm^{-1}): 3347, 3327 (aliphatic NH-stretch bonds - 3288 shoulder bond), 3182 (OH-

stretch bond), 2900 (aliphatic CH-asymmetric stretch bond), 2850 (aliphatic CH-symmetric stretch bond), 1586 (NH-bending), 1287 (CN-stretch bond), and 1206 (C-O stretch bond). N-H asymmetric and symmetric tensile bonds expected around 3550-3420 cm-1 and 3450-3320 cm^{-1} of the aliphatic NH_2 group (Erdik, 2007, p:118) were observed in the 3347-3327 cm^{-1} region. Since the primary amine showed the characteristic doublet structure, it was thought that the metabolites mostly contained the primary NH_2 group. O-H stretch band at the end of aliphatic chains was observed in the region of 3183 cm^{-1}. In addition, the specific diffuse band of the O-H stretch band (Besson et al, 1997) was also observed.

The results of the GC-MS analysis suggested that mostly tromethamine and 1-dodecanol, and some 13-docosenamide were present in the metabolite samples. Accordingly, the asymmetric and symmetric tension and bending vibration bands of CH_2 and CH aliphatic groups, 2900 cm^{-1} and 2850 cm^{-1}, common to the three compounds, were observed in the FT-IR spectra.

In general, when the C=O group is attached to an atom carrying an unshared electron or is conjugated with a double bond, the effect of unshared electron pairs and double bonds in a structure creates the mesomeric effect, while the electron-withdrawing or electron-donating creates the inductive effect. Any factor that increases the dipole character of the C=O group, decreases the value of the force constant, and thus the absorption shifts to the lower frequency (Erdik, 2007, p:151-152). Although the amide C=O stretch band in the 13-docosenamide compound was expected to be observed within the 1650-1700 cm^{-1} region, no band was obvious in this region. In addition, there was also no C=C alkene bands available. These two findings might argue against the presence of 13-docosenamide in the metabolite samples, and it could be considered as the GC-MS artefact, resulting from the compendium of compounds specified by its software. Hence, the FT-IR results confirmed the abundance of two compounds, tromethamine and 1-dodecanol.

The FT-IR spectra of the M2 sample almost overlapped with the M1, except for the CO-stretch bond at 1206 (Fig. 6): *v max*. (cm^{-1}): 3347, 3326 (aliphatic NH-stretch bonds - 3288 shoulder bond), 3186 (OH-stretch bond), 2920 (aliphatic CH-asymmetric stretch bond), 2851(aliphatic CH-symmetric stretch bond), 1587 (C=O stretch bond), 1535 (NH-bending), and 1287 (CN-stretch bond). Here, theoretically, the NH_2 group (Erdik, 2007, p:118), which is expected to be observed around 3550-3420 cm^{-1} and 3450-3320 cm^{-1}, was observed in the 3347-3326 cm^{-1} region with N-H asymmetric and symmetric tension bands. The characteristic doublet structure of the primary amine suggested that the metabolites mostly contain the primary NH_2 group. From these data, it could be said that the metabolite content consisted mostly of tromethamine and 1-dodecanol (Besson et al, 1997).

Discussion

This work constituted our third attempt to demonstrate that different *Streptomyces* species could produce 1-dodecanol as the predominant secondary metabolite under the conditions employed. The presence of tromethamine-like secondary metabolites was, however, unique to this study. Further studies involving the

purification of these compounds by column chromatography and the analysis by NMR spectrometry are required for further characterisations.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) appeared to be a robust and relatively straight forward technique that yielded enough material for analytical purposes. And it was suggested that *n*-butanol could be the organic solvent of choice for the extraction of 1-dodecanol- and tromethamine-like compounds.

Declarations

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interests.

This article does not contain any studies involving animals performed by any of the authors.

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Tables

Table 1
Three predominant compounds of the M1 band (GC-MS analysis)

Peak	Retention time	Name of the compound	Peak (%)
1	26.140	Tromethamine	61.12
2	29.065	1-Dodecanol	17.77
3	46.220	13-Docosenamide	7.71

Table 2
Two predominant compounds of the M2 band (GC-MS analysis)

Peak	Retention time	Name of the compound	Peak (%)
1	29.107	1-Dodecanol	54.06
2	25.650	Tromethamine	41.16

Figures

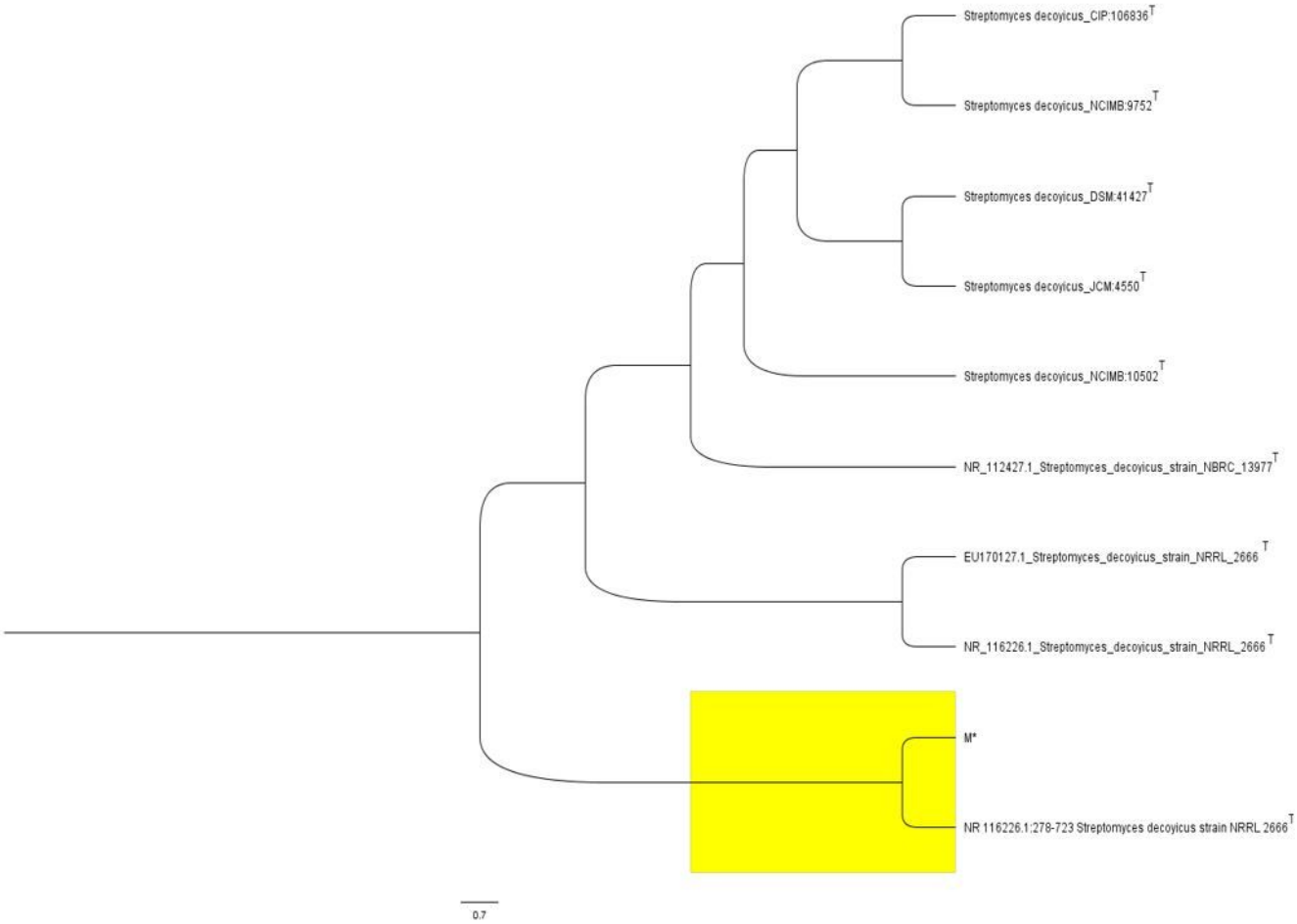

Figure 1

Dendrogram of *Streptomyces decoyicus* type strains and M* isolate.

Figure 2

Thin layer chromatography of the total extract.

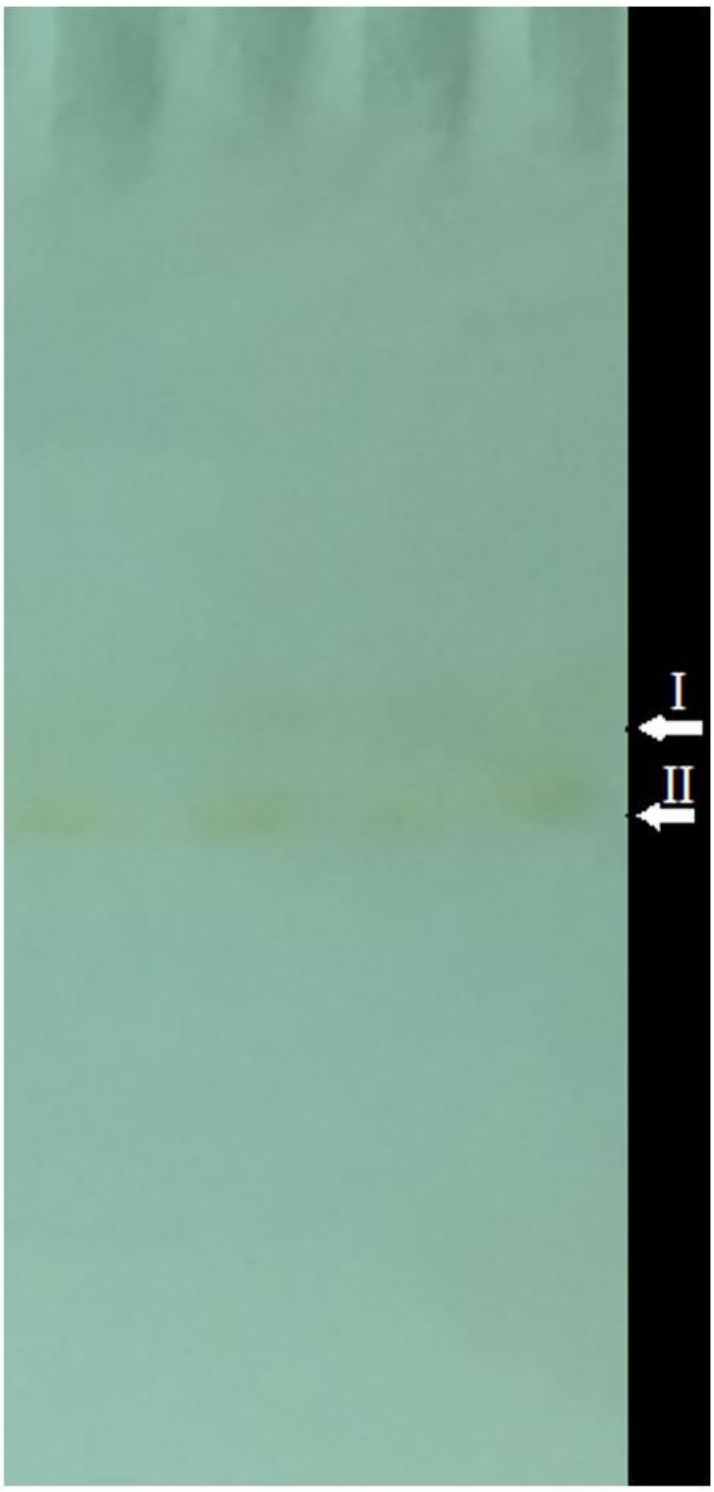

Figure 3

The image of the secondary metabolites obtained from SDS-PAGE;

I: M1 (upper), II: M2 (lower).

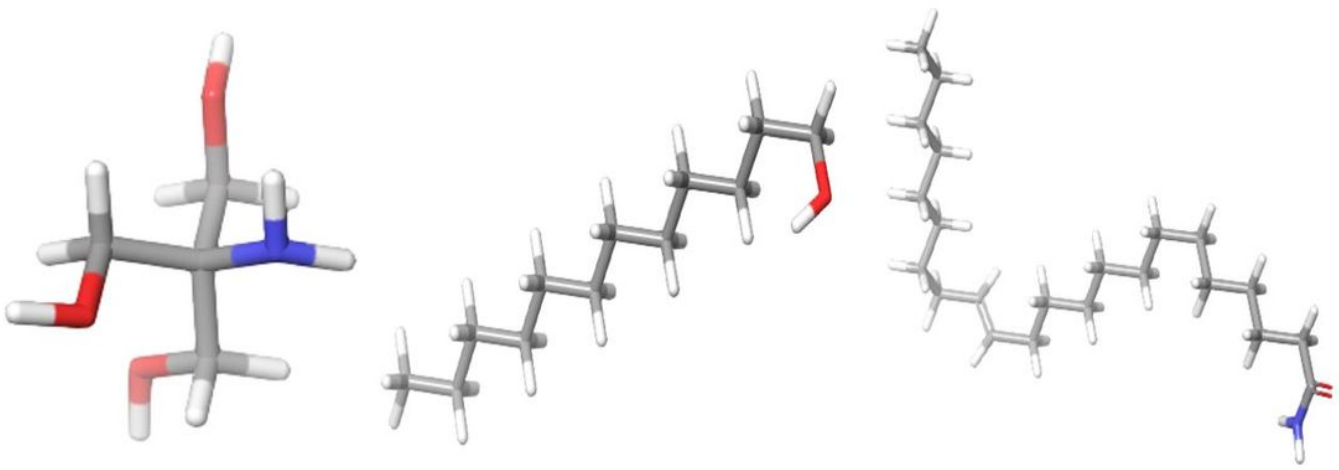

Figure 4

Structure of Molecules; **A:** Tromethamine; **B:** 1-Dodecanol; **C:** 13-Docosenamide.

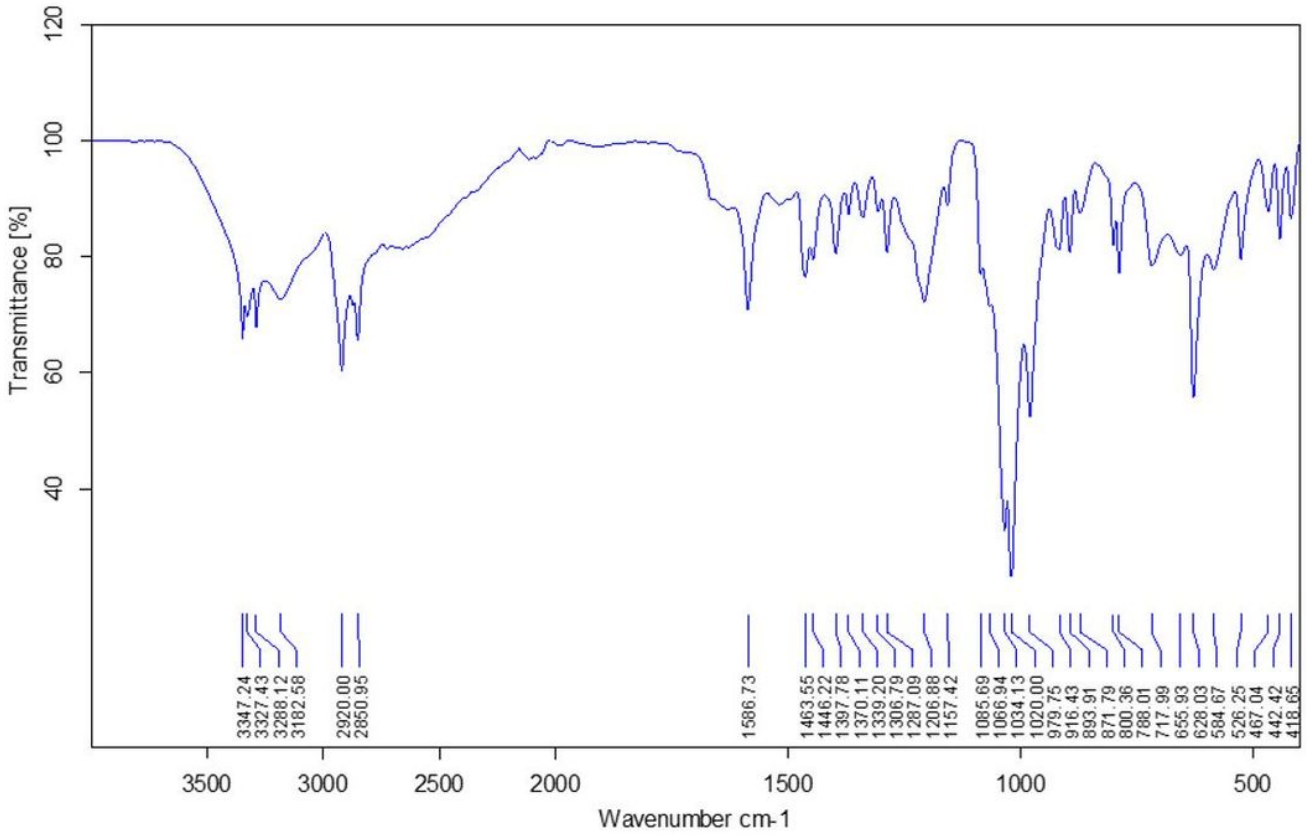

Figure 5

Fourier transform infrared spectra of M1 band content.

Figure 6

Fourier transform infrared spectra of M2 band content.