

## Determination of Phytochemical Constituents, Antimicrobial Activity and Isolation of Secondary Metabolite Compounds from the Stem Barks of *Putranjiva roxburghii* Wall.

(Badi-byu)

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### Abstract

In this research, one of Myanmar indigenous medicinal plant *Putranjiva roxburghii*, popularly known as Badi-byu, was selected for chemical investigation. Firstly, the phytochemical screenings of selected sample were carried out. Moreover, the antimicrobial activities of the sample were determined by Ager-well diffusion method on seven tested organisms with various solvent extracts. Ethyl acetate extract responds high activity on all tested organisms. Hence, it was selected for detailed chemical analysis. Two pure compounds (HWYH-1 and HWYH-2) were isolated from ethylacetate extract of sample by using Thin Layer and Column Chromatographic separation methods. The Fourier Transform Infrared Spectroscopy (FT IR) spectra of isolated compounds (1 and 2) were studied and the prominent functional groups containing in these compounds were assigned.

**Keywords:** *Putranjiva roxburghii*, Badi-byu, phytochemical, antimicrobial, FT IR

### Introduction

Traditional herbal medicines encompass an extremely diverse group of preparations that originate from many different cultures. Generally, herbal products are classified as medicinal products if they claim therapeutic or prophylactic indication, and are not considered as medicinal products when they do not make these claims. Products not classified as medicinal in most cases belong to the food or cosmetic areas, although they sometimes contain plants which have pharmacological properties. (Lyon and France.,2002) Humans have relied on nature for their basic needs for the production of foodstuff, shelters, clothing, means of transportation, fertilizers, flavors, and fragrances, and not the least, medicines. Traditional medicinal plants are readily available and culturally acceptable(Kaliyaperumal Karunamoorthi *et al.*, 2013).

*Putranjiva roxburghii* also called as Putranjiva or Putrajeevak a well-known moderate sized, evergreen tree growing up to 12 m in height. It is widely grown in Thailand, Nepal, Bangladesh, India, Myanmar and Sri Lanka. In folklore medicine, its leaves and fruits have been traditionally

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used for the treatment of fever, muscle twisting, arthralgia and rheumatism. They have also been used as anti-nociceptive, antipyretic and anti-inflammatory while the whole plant of *P.roxburghii* has been used for the treatment of fever and haemorrhoids. (Mradu Gupta., 2016) Putranjiva can be used as Biofuel, Herbal preservative, Trypsin Inhibitor, Antifungal, Antipyretic and Anti-diabetic agent. Further researches can be taken over this plant especially in its stem bark as the extracts may have an extraordinary medicinal or commercial value within. (Supriya B. *et al.*,2017)

### Botanical Description

Botanical name	-	<i>Putranjiva roxburghii</i> Wall.
Family name	-	Putranjivaceae
Species	-	<i>roxburghii</i>
English name	-	Lucky bean
Myanmar name	-	Badi-byu, Ye-padi
Part uses	-	Stem barks
Medicinal uses	-	Hypoglycaemic, anti-nociceptive, antipyretic, anti-inflammatory, cytotoxic, antioxidant, antimicrobial activities and anti-diabetic agent.

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**Figure 1. Plant and stem barks of *Putranjiva roxburghii***

### Aim

The aim of present research is to investigate the preliminary phytochemical screenings, the antimicrobial activity on crude extracts, extraction and isolation of pure compounds from the stem barks of *Putranjiva roxburghii*.

### Materials and Methods

In the isolation and purification of pure compounds, common laboratory apparatus, Column and Thin Layer Chromatographic methods were used. Commercial grade reagents and solvents such as ethyl acetate, n-hexane and ethanol were rectified by distillation before they were used in the experiment. Column chromatography was carried out on silica gel (70-230) mesh. Analytical preparative Thin Layer Chromatography was performed by using Kieselgel 60 (F<sub>254</sub>, Merck). In Thin Layer Chromatography, visualization was taken via UV lamp (Lambda- 40, Perkin- Elmer Co,

Japan) and iodine vapor was used as color development. Crude and purified extracts were weighed in Electric Balance (Shimadzu, Japan).

### **Collection and Preparation of Samples**

The stem barks of *Putranjiva roxburghii* were collected from Ngazun Township, Mandalay Region and identified by the Department of Botany, University of Mandalay. Firstly, the samples were cleaned, then chopped into small pieces and allowed to air dry in the well ventilated room for about two weeks. These air dry pieces of sample were kept in the glass bottle with stopper and they were used throughout the experiment.

#### **A. Preliminary Phytochemical Analysis**

The phytochemical investigation of the samples were carried out by standard method (Harbone, 1984). Preliminary phytochemical analysis for alkaloid, flavonoid, glycoside, lipophilic, phenolic, polyphenol, reducing sugar, saponin, tannin, steroid, and terpene weretested and each test was quantitatively expressed as negative (-) or positive (+).

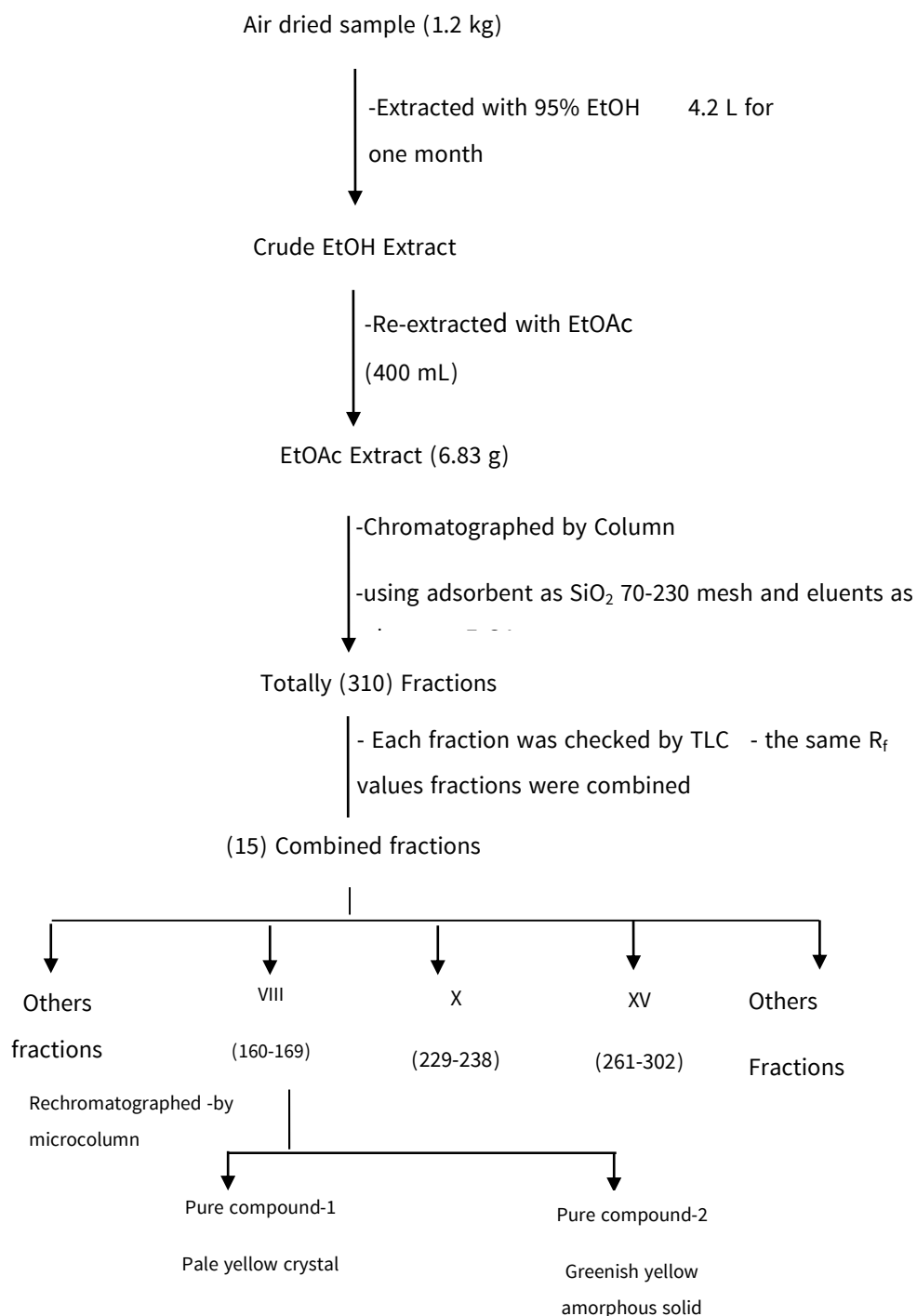
### **Antimicrobial Activities of the Stem Barks of *Putranjiva roxburghii***

The microorganisms used for the antimicrobial activity screening were *Baccillus subtills*, *Staphylococcus aureus*, *pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican*, *E.coli* and *Agrobacterium tumefaciens*. Antimicrobial activities of this selected medicinal plant were tested in various solvent systems by using Agar-well diffusion method in Department of Chemistry, Meiktila University, Meiktila.

### **Extraction and Isolation of the StemBarks of *Putranjiva roxburghii***

1.2kg of air dried sample was extracted with 95% ethanol (4.2L) for one month. Ethanol extract was filtered and evaporated at room temperature. It was re-extracted with ethylacetate (400mL) and ethylacetate crude extract (6.83 g) was chromatographed by column. Pure compounds (1 and 2) were isolated from the ethylacetate extract of selected sample by using Thin Layer and Column Chromatographic separation methods. The extraction and isolation procedure were described in following flowsheet.

### Flowsheet for Extraction and Isolation of the Stem Barks of *Putranjiva roxburghii*



#### FT IR Measurement of Pure compounds

The FT IR spectra of pure compounds (1 and 2) were measured at the Department of Chemistry, University of Mandalay. The spectra were collected by a SHIMADZU (Japan) FT IR-410 spectrophotometer. The prominent functional groups containing in isolated compounds were assigned.

### Results and Discussion

This section consists of the results of the experimental works such as preliminary phytochemical screenings, antimicrobial activity of various solvent extracts, isolation of pure compounds, FT IR spectra of isolated pure compounds and their assignments.

#### Preliminary Phytochemical Screenings of *Putranjiva roxburghii*

Preliminary phytochemical screenings of the stem barks of *Putranjiva roxburghii* were examined and results were tabulated in Table 1.

Table 1. Results of Phytochemical Tests for Barks of *Putranjiva roxburghii*

No.	Constituents	Reagents used	Observation	Results
1	Glycoside	10% lead acetate	White ppt	+
2	Phenolic	10% FeCl <sub>3</sub>	Dark green color solution	+
3	Reducing sugar	Benedict's solution	Red color solution	+
4	Lipophilic	0.5 M KOH, 0.1 M NaOH	Deep color solution	+
5	Saponin	Distilled water, shake	Frothing	+
6	Flavonoid	EtOH, Conc: HCl, Mg turnings	Pink color solution	+
7	Alkaloid	Drangendorff's reagent Wagner's reagent	Yellow ppt Brown ppt	+
8	Steroid	Pet-ether, Acetic anhydride, Conc: H <sub>2</sub> SO <sub>4</sub>	Blue color solution	+
9	Terpene	CHCl <sub>3</sub> , Acetic anhydride, Conc: H <sub>2</sub> SO <sub>4</sub>	Pink color solution	+
10	Polyphenol	1% FeCl <sub>3</sub> , 1% K <sub>3</sub> Fe(CN) <sub>6</sub>	Greenish blue ppt	+
11	Tannin	10% FeCl <sub>3</sub>	Pale brown color solution	+

(+) = presence, (-) = absence, ppt = precipitate

According to this table, the stem barks of *Putranjiva roxburghii* consist of glycoside, phenolic, reducing sugar, lipophilic, saponin, flavonoid, alkaloid, steroid, polyphenol, tannin and terpene respectively.

#### Determination of Antimicrobial Activities of *Putranjiva roxburghii*

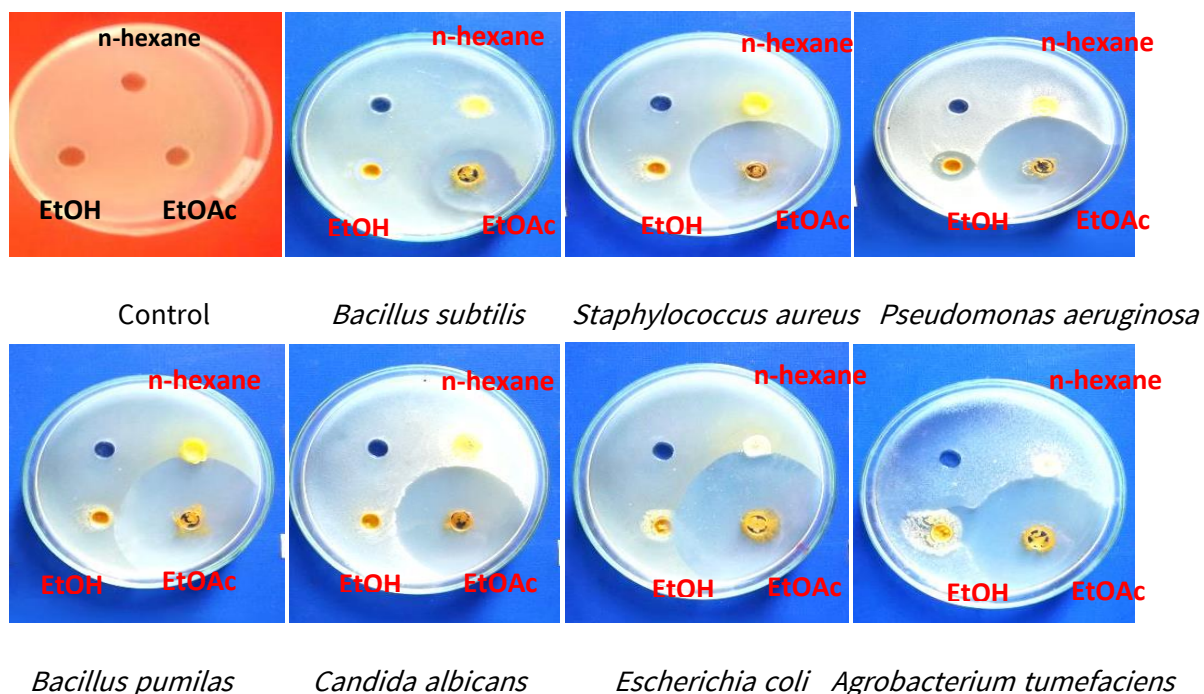
The results of antimicrobial activities of the crude sample were shown in table 2 and figure 2. According to this table, the ethyl acetate extract of the stem barks of *Putranjiva roxburghii* showed high activities on all tested organisms. But, Ethanol extract gave medium activities on, *Candida albican* and *Agrobacterium tumefaciens*. Furthermore, n-hexane extract did not show antimicrobial activities on all tested organisms.

**Table 2. Antimicrobial Activities of the StemBarks of *Putranjiva roxburghii***

Sample	Solvents	Inhibition Zone Diameter						
		I	II	III	IV	V	VI	VII
<i>Putranjiva roxburghii</i> Wall.	n-hexane	-	-	-	-	-	-	-
	EtOAc	51 mm (+++)	26 mm (+++)	47 mm (+++)	58 mm (+++)	49 mm (+++)	56 mm (+++)	51 mm (+++)
	EtOH	-	-	-	10 mm (++)	-	-	16mm (++)

**Organisms**

- I = *Bacillus subtilis* (N. C. T. C - 8236) Agar well – 10 mm  
 II = *Staphylococcus aureus* (N.C.P.C - 6371) 10 mm~14 mm (+)  
 III = *Pseudomonas aeruginosa* (6749) 15 mm~19 mm (++)  
 IV = *Bacillus pumilus* (N.C. I. B - 8982) 20 mm above (+++)  
 V = *Candida albicans*  
 VI = *E-coli* (N.C.I.B - 8134)  
 VII = *Agrobacterium tumefaciens*

Figure 2. Antimicrobial activities of the stem barks of *Putranjiva roxburghii***Isolation and Purification of Pure Compounds**

Two pure compounds (HWYH-1, 53.2 mg, 0.7789% yield) and (HWYH-2, 32.1 mg, 0.4699% yield) were isolated from the stem barks of *Putranjiva roxburghii*. Pure yellow crystal compound (HWYH-1) gave only one spot on TLC and  $R_f$  value is 0.45 with solvent system n-hexane: ethyl acetate (2:3 v/v). And then, pure compound (HWYH-2), greenish yellow amorphous solid

compound, was also only one spot on TLC,  $R_f$  value 0.25 with the solvent ratio of n-hexane : ethyl acetate (2:3 v/v).

### C. FT IR Assignments of Pure Unknown Compound (HWYH-1)

The FT IR spectrum of pure compound (HWYH-1) was shown in Figure 3. In this spectrum, the broad band which appeared at  $3494.20\text{ cm}^{-1}$  indicated the O-H stretching vibration of alcohol group. The peak at  $3030.30\text{ cm}^{-1}$  indicated  $sp^2$  hydrocarbon. The peak at  $2948.32\text{ cm}^{-1}$  and  $2855.73\text{ cm}^{-1}$  indicated asymmetric and symmetric C-H stretching vibration of  $sp^3$  hydrocarbon. The peak at  $1704.18\text{ cm}^{-1}$  showed C=O stretching vibration of carbonyl group. The peak at  $1603.88\text{ cm}^{-1}$  showed the C=C ring skeletal stretching vibration of aromatic benzene ring. The band at  $1461.14\text{ cm}^{-1}$  indicated the C-H in plane bending vibration of allylic hydrocarbon. The peak at  $1381.09\text{ cm}^{-1}$  showed the C-H stretching vibration of gem dimethyl group. On the other hand,  $1275.00\text{ cm}^{-1}$  is assumed to be C-C-O stretching vibration of alcohol group. The peak at  $1168.91\text{ cm}^{-1}$ ,  $1112.97\text{ cm}^{-1}$  and  $1035.82\text{ cm}^{-1}$  showed the C-O-C stretching vibration of ether group. The band at  $979.88\text{ cm}^{-1}$  is assumed to be =C-H out of plane bending vibration of trans or E alkenic group. Finally, the band at  $812.07\text{ cm}^{-1}$  was due to =C-H out of plane bending vibration of cis or Z alkenic group. According to FT IR spectrum, pure compound HWYH-1 should consist of alcohol group,  $sp^2$  hydrocarbon,  $sp^3$  hydrocarbon, carbonyl group, allylic hydrocarbon, gem dimethyl group, ether group, trans or E alkenic group and cis or Z alkenic group respectively. The functional groups observed in FT IR spectrum of pure compound (HWYH-1) are tabulated in Table 3.

**Table 3.** FT IR Assignments of Pure Compound (HWYH-1)

No.	Frequencies ( $\text{cm}^{-1}$ )	Assignments
1	3494	O-H stretching vibration of alcohol group
2	3030	C-H stretching vibration of $sp^2$ hydrocarbon
3	2948, 2855	Asymmetric and symmetric C-H stretching vibration of $sp^3$ hydrocarbon
4	1704	C = O stretching vibration of carbonyl group
5	1603	C = C ring skeletal stretching vibration of aromatic benzene ring
6	1461	C-H in plane bending vibration of allylic hydrocarbon
7	1381	C-H stretching vibration of gem dimethyl group
8	1275	C-C-O stretching vibration of alcohol group
9	1168, 1112, 1035	C-O-C stretching vibration of ether group
10	979	= C-H out of plane bending vibration of trans or E alkenic group
11	812	= C-H out of plane bending vibration of cis or Z alkenic group

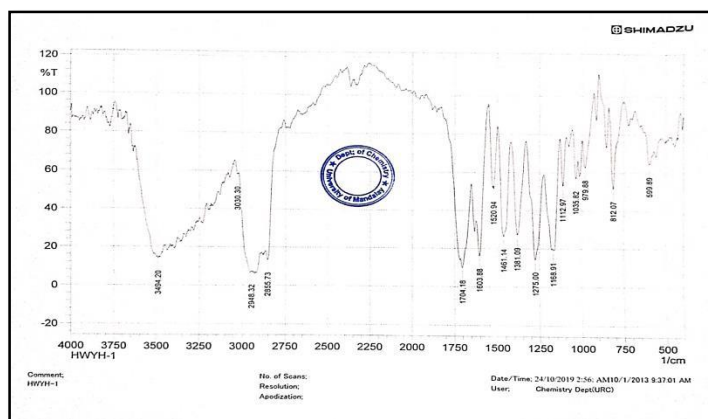


Figure 3. FT IR spectrum of isolated pure compound (HWYH-1)

#### A. FT IR Assignments of Isolated Pure Compound (HWYH-2)

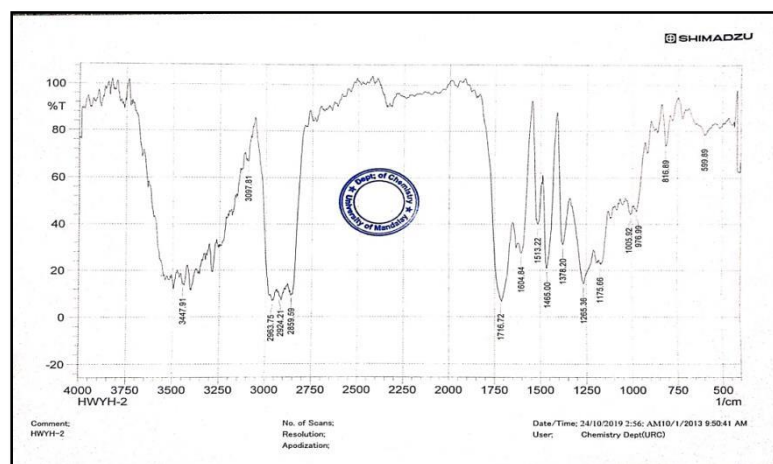
The FT IR spectrum of pure compound (HWYH-2) was shown in Figure 4. In this spectrum, the broad band which appeared at  $3447\text{ cm}^{-1}$  indicated the O-H stretching vibration of alcohol group. The peak at  $3097\text{ cm}^{-1}$  indicated  $\text{sp}^2$  hydrocarbon. The peak at  $2963$ ,  $2924\text{ cm}^{-1}$  and  $2859\text{ cm}^{-1}$  indicated asymmetric and symmetric C-H stretching vibration of  $\text{sp}^3$  hydrocarbon. The peak at  $1716\text{ cm}^{-1}$  showed C=O stretching vibration of carbonyl group. The peak at  $1604\text{ cm}^{-1}$  showed the C=C ring skeletal stretching vibration of aromatic benzene ring. The band at  $1465\text{ cm}^{-1}$  indicated the C-H in plane bending vibration of allylic hydrocarbon. The peak at  $1378\text{ cm}^{-1}$  showed the C-H stretching vibration of gem dimethyl group. Furthermore,  $1265\text{ cm}^{-1}$  is assumed to be C-C-O stretching vibration of alcohol group. The peak at  $1175\text{ cm}^{-1}$  and  $1005\text{ cm}^{-1}$  showed the C-O-C stretching vibration of ether group. The band at  $976\text{ cm}^{-1}$  is assumed to be =C-H out of plane bending vibration of trans or E alkenic group. Finally, the band at  $816\text{ cm}^{-1}$  was due to =C-H out of plane bending vibration of cis or Z alkenic group. According to FT IR spectrum, pure compound HWYH-2 should consist of alcohol group,  $\text{sp}^2$  hydrocarbon,  $\text{sp}^3$  hydrocarbon, carbonyl group, allylic hydrocarbon, gem dimethyl group, ether group, trans or E alkenic group and cis or Z alkenic group respectively. The functional groups observed in FT IR spectrum of pure compound (HWYH-2) are tabulated in Table 4.

Table 4. FT IR Assignments of Pure Compound (HWYH-2)

No.	Frequencies ( $\text{cm}^{-1}$ )	Assignments
1	3447	O-H stretching vibration of alcohol group
2	3097	C-H stretching vibration of $\text{sp}^2$ hydrocarbon
3	2963, 2924, 2859	Asymmetric and symmetric C-H stretching vibration of $\text{sp}^3$ hydrocarbon
4	1716	C=O stretching vibration of carbonyl group
5	1604	C=C ring skeletal stretching vibration of aromatic benzene ring



6	1465	C-H in plane bending vibration of allylic hydrocarbon
7	1378	C-H stretching vibration of gen dimethyl group
8	1265	C-C-O stretching vibration of alcohol group
9	1175,1005	C-O-C stretching vibration of ether group
10	976	= C-H out of plane bending vibration of trans or E alkenic group
11	816	= C-H out of plane bending vibration of cis or Z alkenic group



**Figure 4.** FT-IR spectrum of isolated pure compound (HWYH-2)

### Conclusion

In this research, the stem barks of *Putranjiva roxburghii* were chosen for chemical investigations. As described in Table 1, the bark of *Putranjiva roxburghii* contained various chemical constituents such as alkaloid, flavonoid, glycoside, lipophilic, phenolic compound, polyphenol, reducing sugar, saponin, steroid, tannin and terpene compounds.

As shown in Table 2, the antimicrobial activities of various solvent extracts of the stem bark of *Putranjiva roxburghii* were tested by Agar-well diffusion method on seven selected organisms. Ethylacetate extract of *Putranjiva roxburghii* responds high activities on all tested organisms. Pure compounds (HWYH-1) (53.2 mg, 0.7789% yield) pale yellow crystal and (HWYH-2) (32.1 mg, 0.4699% yield) greenish yellow amorphous solid were isolated from the ethylacetate extract of selected plant by using Thin Layer and Column Chromatography method. The FT IR spectra of isolated compounds were measured and the functional groups containing in these isolated compounds were assigned.

According to FT IR spectrum, pure compound (HWYH-1) consisted of alcohol group,  $sp^3$  hydrocarbon,  $sp^2$  hydrocarbon, carbonyl group, aromatic benzene ring, allylic hydrocarbon, gen dimethyl group, C-C-O stretching vibration of alcohol group, ether group, trans or E and cis or Z alkenic groups. The FT-IR spectrum of pure compound (HWYH-2) informed the presence of alcohol group,  $sp^3$  hydrocarbon,  $sp^2$  hydrocarbon, carbonyl group, aromatic benzene ring, allylic

hydrocarbon, gem dimethyl group, C-C-O stretching vibration of alcohol group, ether group, trans or E and cis or Z alkenic groups respectively. The structure elucidation of these compounds will be assigned in future.

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