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# *In vitro* Antimicrobial Effect of three Terpenes, Isolated from the Bark of *Zanthoxylum budrunga*

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**Abstract:** Three terpenes lup-20 (29)-en-3-one (ZB-1), 11β, 13-dihydro-1epireynosin (ZB-2) and tetracyclic diterpenol-15-cinnamate (ZB-3) were isolated from the bark of the plant *Zanthoxylum budrunga* and were screened against some pathogenic bacteria and fungi. They were found significant *in vitro* antibacterial and antifungal. The zones of inhibition produced by the pure compounds were laid between 9 mm to 23 mm. The MIC values of the three isolated compounds were also determined against four test organisms ($10^7$ cells/ml) and were found to be effective between 16 μg/ml to 128 μg/ml.

**Key words:** *Z. budrunga*, antibacterial, antifungal, terpenes

## Introduction

*Zanthoxylum budrunga*, locally known as "Bajna" in Bangladesh, belongs to family Rutaceae (Parin, 1963). It is revealed that most of the plants belonging to this family possess secondary metabolites of medicinal importance (Uphof, 1968). The fruit of *Z. budrunga* is used in asthma, bronchitis, heart diseases, piles dysentery and in rheumatism with honey (Kirtikar and Basu, 1993). The essential oil of leaves is used for cholera and juice of bark cures dysentery, cough, headache and vomiting (Kirtikar and Basu, 1993).

Some chemical works have been carried out on the seeds, leaves and roots of the plant *Z. budrunga*. Four volatile compounds, β-phelalndrenr, hydroxy α-sanshool, pipertone, β-pinrne (Tirilline *et al.*, 1994), two alkaloids, arborine, diatamnine (Ruangrungsi *et al.*, 1981) and a rutaecarpine (Benerjee *et al.*, 1989) have been isolated from the fruits. A new monoterpene triol, trihydroxy-p-menthane was also isolated from the root (Thappa *et al.*, 1976). However, there was few reports on chemical and biological works on the bark of the plant. Therefore, our attention was concentrated on the bark of *Z. budrunga*.

In this paper, we report the isolation of three terpenes from the bark of *Z. budrunga* and their antimicrobial investigation.

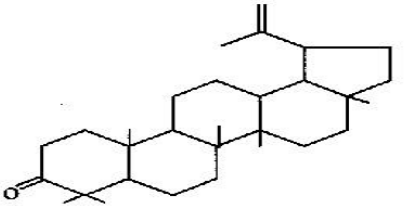
## Materials and Methods

**Plant materials:** Matured bark was collected from the Tangail Hill of Bangladesh, in October, 1997 and identified by Prof. N. Zaman, Department of Botany, University of Rajshahi, where a voucher specimen has been deposited. The bark was then cut into small pieces, dried in a oven at 40°C to a constant weight and pulverized into fine powder by a grinding machine, then stored in air tight container

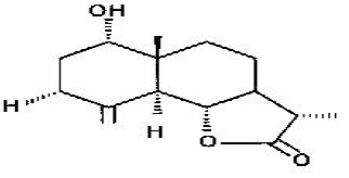
**Extraction, isolation and characterization:** The plant material (1kg) was extracted with petroleum ether, chloroform, and ethyl acetate in Soxhlet apparatus. Each of the solvent was filtered with Whatman No. 1 filter paper and concentrated at 50°C under reduced pressure using a rotary evaporator to afford a semi solid mass individually.

Three terpenes ZB-1, ZB-2 and ZB-3 were isolated from the chloroform extract (24.5 gm) by column chromatography (Beckett and Stenlake, 1986) eluting with petroleum ether and ethyl acetate followed by TLC and PTLC (Egon and Stahl, 1969). The silica of respective bands were scraped off from the plates (20 cm × 20 cm) and washed by ethyl acetate assisted with sanitation. The percentages of yield of ZB-1, ZB-2 and ZB-3 were 0.54%, 0.78% and 0.36%, respectively.

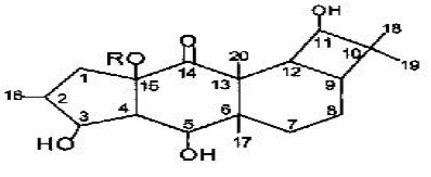
All these compounds were characterized on the basis of their UV. IR, NMR and Mass spectral data and comparison with previously reported values (Prashant *et al.*, 1993; Uemura *et al.*, 1976 and Adof *et al.*, 1984). Then all the three compounds were subjected to antibacterial and antifungal screening.



**ZB-1: Lup-20(29)-en-3-one**



**ZB-2: 11β, 13-dihydro-1-epireynosin**



**ZB-3: Tetracyclic diterpenol-15-cinnamate**

**Antibacterial screening:** Twelve pathogenic bacteria (5 gram positive and 7 gram negative) were selected for the test and collected from the Department of Microbiology, Dhaka University, Dhaka, Bangladesh. Nutrient agar was used as bacteriological media.

All three isolated compounds were dissolved separately in sufficient volume of methanol to get a concentration of 200 µg per 10 µl. Then *in vitro* antibacterial activity of these compounds were carried out by the standard disc diffusion method (Barry, 1980; Berghe and Vlietuck, 1991; Beur *et al.*, 1966 and Rios *et al.*, 1988) against selected test organisms. The diameters of zone of inhibition produced by the compounds were compared with those produced by the standard antibiotic (kanamycin 30 µg/disc). The experiment was performed in duplicate to minimize the errors.

**Minimum Inhibitory Concentration (MIC):** The MIC values of three isolated compounds were determined against three gram positive (*B. cereus, B. subtilis* and *Staphylococcus aureus*) and two gram negative (*E. coli* and *Sh. dysenteriae*) bacteria. The test was carried out by serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

**Antifungal screening:** Five pathogenic fungi were selected for the test and collected from the Department of Botany, University of Rajshahi, Bangladesh. PDA was used as fungicidal media. All three compounds were dissolved separately in sufficient volume of methanol to get a concentration of 400 µg/ disc. Then *in vitro* antifungal activities of these compounds were performed by disc diffusion method (Beur *et al.*, 1966). Methanol was used as negative or blank control in this experiment.

## Results and Discussion

The antibacterial activity of three terpenes against twelve pathogenic bacteria is presented in Table 1. It is clearly that all the isolated compounds showed significant antibacterial activity against almost all test bacteria. The compound ZB-1, ZB-2 and ZB-3 produced zone of inhibition between 9-19 mm, 12-23 mm and 11-21 mm, respectively.

Among the terpenes, compound ZB-2 showed highest inhibitory activity towards almost all bacteria and in particularly against *B. megaterium*, *E. coli* and *Shigella shiga*. The MIC values of all the isolated compounds on five-test organisms are presented in Table 3 and it is evident that ZB-1 showed comparatively good effect.

Table 1: Antibacterial activities of ZB-1, ZB-2 and ZB-3 isolated form bark of *Z. budrunga*

| Test organism | ZB-1 | ZB-2 | ZB-3 | ZB-4 |
|---|---|---|---|---|
| **Gram positive** | | | | |
| *Bacillus cereus* | 15 | 16 | 21 | 24 |
| *Bacillus subtilis* | 19 | 15 | 16 | 26 |
| *Bacillus megaterium* | - | 14 | 13 | 28 |
| *Staphylococcus aureus* | 19 | 15 | - | 27 |
| *Streptococcous β haemolyticus* | 9 | - | 14 | 24 |
| **Gram negative** | | | | |
| *Escherichia coli* | 18 | 23 | 15 | 27 |
| *Shigella dysenteriaae* | 14 | 12 | 16 | 26 |
| *Shigella shiga* | - | 15 | 11 | 22 |
| *Shigalla flexneriae* | 15 | 14 | 15 | 27 |
| *Sigella sonnei* | - | - | - | 23 |
| *Shigella boydii* | 11 | 13 | 14 | 25 |
| *Klebsiella* species | 10 | 14 | 12 | 19 |

ZB-1 =Lup-20 (29) -en-3-one (200µg/disc)
ZB-2 =11β, 13-dihydro-1 epireynosin (200µ/disc)
ZB-3 =Tetracyclic diterpenol-15-cinnamate (200µg/disc)
SA = Kanamycin (30µg/disc) - = no sensitivity

Tabel 2: Antifungal activities of the compnds ZB-1, ZB-2 and ZB-3 isolated from bark of *Z. Budrunga*

| Total organism | ZB-1 | ZB-1 | ZB-1 |
|---|---|---|---|
| *Aspergillus fumigatus* | 13 | 12 | 13 |
| *Hensinela californica* | 11 | 14 | 10 |
| *Rhizopus orizae* | 11 | 12 | 15 |
| *Schizosporum* species | 15 | 10 | 13 |
| *Rhizopus oligosporum* | 11 | 12 | 13 |

Table 3: The MIC values of the isolated compounds against test organism

| Minimum inhibitory concentration in µg/ml - | Isolated compounds | | |
|---|---|---|---|
| | ZB-1 | ZB-2 | ZB-3 |
| *B. cereus* | 64 | 128 | 16 |
| *B. subtilis* | 32 | 128 | 64 |
| *Sta.aureus* | 64 | 128 | - |
| *E.coli* | 32 | 64 | 64 |
| *Sh. dysenteriae* | 128 | - | 128 |

ZB-1 =Lup-20 (29) -en-3-one
ZB-2 =11β, 13-dihydro-1 epireynosin
ZB-3 =Tetracyclic diterpenol-15-cinnamate
- = no sensitiviy

Moreover, from the experimental results of antifungal activity, presented in Table 2, it is revealed that all the isolated compounds showed significant activity against five pathogenic plant fungi. However, ZB-3 showed highest activity towards all test fungi.

In conclusion, the present study reports for the first time the antibacterial and antifungal activity of three terpenes isolated form the bark of *Z. budunga*. However, further and specific studies are needed to better evaluate the potential effectiveness of the three isolated terpenes from the bark of *Z. budrunga* as an anitmicrobial agent.

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