

# Extraction and Thin Layer Chromatography of Alkaloids from Bael (*Aegle marmelos*) Leaves

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Article Info	Abstract
<b>Article History</b> Received : 25-03-2011 Revised : 17-04-2011 Accepted : 20-04-2011	Bael tree, ( <i>Aegle marmelos</i> , Family- Rutaceae), is a sacred tree widely grown in Indian forests, Ceylon, Burma, Thailand and Indo-China. All parts of the tree are used for medicinal purpose. Some of the medicinal properties are astringent, antidiarrheal, antidysenteric, demulcent, stromachic, fever curing, insulin-promoter, anti-inflammatory, cardio tonic and cures ophthalmic, urinary trouble, palpitation and many more. Most of these properties are believed to be due to presence of bioactive alkaloids in Bael. This study was carried out to develop an easy method of extraction and separation of alkaloids from dried Bael leaves. Alkaloids from the dried leaves of Bael were extracted in 95% ethanol and separated with chloroform in presence of synthetic antioxidants butylated hydroxytoluene (BHT and butylated hydroxyanisole (BHA) by thin layer chromatography. BHT and BHA seem to protect alkaloids from their degradation/ conversion to other components.
<b>*Corresponding Author</b> Tel : +91 9970419218  Email: avigoplani1@rediffmail.com	<b>Key Words:</b> Bael, <i>Aegle marmelos</i> , Alkaloids, Extraction, TLC, BHT, BHA, Antioxidants
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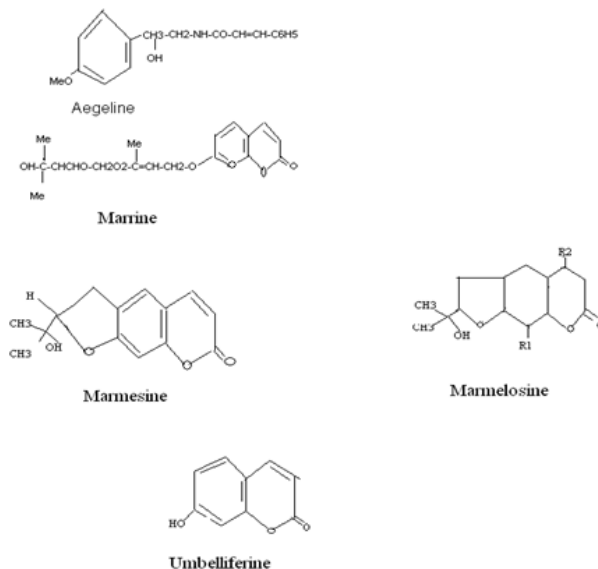
## Introduction

Bael is a sacred tree from India, of Rutaceae family, related to citrus. It is a beautiful medium size tree (average is 8.5 m tall), with spines on its branches and very aromatic. Leaves are pale green and trifoliate. Flowers are greenish white, sweetly scented, fruits are yellowish green. It is a good source of vitamin C and protein (1).

There are several medicinal uses of Bael in curing diarrhea, fever, poor absorption, and bleeding, vomiting,

nausea with blood, bronchitis, and gingivitis. Decoction of leaves is febrifuge, expectorant, asthmatic complaints (1). The leaves contain many constituents like alkaloids, aegeline, alkaloid coumarine, and marmine, sterol sitosterol, and essential oils d-limonene (1).

The structures of few known alkaloids present in leaves are:-(3)



Aegeline has recently attracted the interests of several researches (2-9). However, we could not identify simple

methods for extraction and thin layer chromatography of Aegeline and other alkaloids from Bael leaves. This paper

describes a method for the extraction and separation of alkaloid that are present in the Bael leaves. We have also studied the effect of addition of BHA and BHT on the degradation/ conversion of alkaloids to other components.

**Materials and Methods**

The plant material required for the study was fresh. Tender leaves were harvested as it has 0.15-0.2% yields from the month of November to March (2). These leaves were kept in sunlight and the dry leaves were powdered and stored.

TLC plates 60F 254 were obtained from Merck. Solvents and other chemicals used were of laboratory grade.

**Extraction method**

Powder of 40 gm of dried leaves was added to 150 ml of 95% ethanol, heated at 65°C and kept overnight at room temperature. The extract was then filtered and again 150 ml of 95% ethanol was added and procedure was repeated one more time for extraction of alkaloids from the leaves. The combined extract was allowed to dry (10). A greenish viscous extract was obtained.

**Separation method**

Analytical and preparative thin layer chromatography was performed on silica gel plates (60 F 254 or glass plates coated with silica gel G 60) with chloroform as solvent system. In some experiments BHT and BHA (0.01gm/ml) was added to

chloroform. Chromatograms were visualized under ultra violet light.

**Effect of addition of BHT ant BHA**

The extract was stored in vials in refrigerator. However, during chromatography and subsequent chromatograms, it was observed that the number of bands slowly increased with time. The appearance of new bands was suspected to be due to oxidation. To avoid oxidation, we added BHT or BHA to some samples.

**Results**

**Extraction and Thin layer chromatography**

Ethanol extract obtained from Bael leaves was carried out to thin layer chromatography in order to establish the purity and composition of materials. The purified components of ethanol extract of Bael leaves were identified by iodine test. 10 bands were observed under ultraviolet light. The Rf values of separated components of Bael leaves extract were measured. Band number 1 was observed at Rf =0.045. Band number 2 was observed at Rf=0.072. Band number 3 was observed at Rf=0.109. Band number 4 was observed at Rf= 0.136. Band number 5 was observed at Rf= 0.236. Band number 6 was observed at Rf= 0.481. Band number 7 was observed at Rf=0.581. Band number 8 was observed at Rf =0.636. Band number 9 was observed at Rf =0.80. Band number 10 was observed at Rf= 0.909. (Table 1) (Fig: 5).

Table 1: Rf values of spots obtained on gel plate without addition of antioxidants

Distance traveled by compound	Rf values of each compound
1) 0.5	0.045
2) 0.8	0.072
3) 1.2	0.109
4) 1.5	0.136
5) 2.6	0.236
6) 5.3	0.481
7) 6.4	0.581
8) 7.0	0.636
9) 8.8	0.8
10) 10.0	0.909

Table 2: Rf values of spots obtained on gel plate with addition of antioxidants BHT

Distance traveled by compound	Rf value of each compound
1) 0.1	0.01
2) 0.4	0.05
3) 1	0.125

Table 3: Rf values of spots obtained on gel plate with addition of antioxidants BHA

Distance traveled by compound	Rf value of each compound
1) 0.1	0.01
2) 0.4	0.05
3) 1	0.125
4) 4.4	0.55
5) 4.9	0.612
6) 6.8	0.85

Table 4: Rf values of the samples

Distance traveled by compound	Rf values of each compound
1) 0.1	0.01
2) 0.4	0.05
3) 1	0.125

**Effect of addition of BHT and BHA**

When BHT and BHA were added, oxidation/ degradation stopped and the mobility of compound also increased. The photographs of gel plates showing the bands that are separated on addition of BHT and BHA. (Fig 1, 2, 3). The Rf values of the bands obtained on the gel plate on addition of BHT and BHA shows its effect on the sample (Table: 2).



Fig.1: Spots of TLC plate containing BHT, BHA and sample seen under UV light



Fig 2: Bands observed on TLC plate Containing BHT, BHA and sample



Fig. 3:Bands obtained on addition of BHT, BHA and sample



Fig.4:Bands to obtain Rf values of BHT and BHA

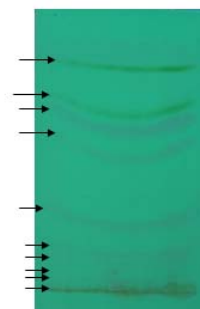


Fig.5: Bands obtained on gel plate without addition of antioxidants.

**Result and Discussion**

The alkaloids present in the leaves are well extracted through 95% ethanol. During separation, it was observed that 10 bands were separated and on recurrent performance of TLC, the samples used to get oxidized which simultaneously affected the mobility of bands, which created the need to stop rancidity of compound. When gel plate containing synthetic antioxidants in its sample were observed under UV light, it showed that on addition of BHT, the degradation of compound stopped while on addition of BHA, there was no effect on degradation/rancidity of compound.

We made this observation through the green bands that were seen on the TLC plate. The addition of synthetic antioxidants like BHT and BHA helped in separation as it protects the compound from getting oxidized and further more the bands of BHT and BHA can be removed by knowing its Rf values and the alkaloids are separated successfully by thin layer chromatography technique.

This extraction and separation method will be useful further to study more medicinal properties of the alkaloids that are present in the extract of Bael leaves.

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