

**QUALITATIVE EVALUATION OF MARKET SAMPLES OF
UNRIPENED FRUIT OF BILWA (AEGLE MARMELLOS CORR.) BY
THIN LAYER CHROMATOGRAPHY**

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ABSTRACT

In present era, even with the advancements in the modern medicine and discovery of effective drugs of synthetic origin in the recent past, the indigenous, traditional medicines still remain alive as precious cultural heritage in different societies of the world. These continue to cater in a significant matter to the medicinal needs of the third world countries which are inhabited by about 80 percent world's population. However, there are a lot of concerns about the traditional medicine in areas of quality, safety and efficacy. Hence, Standardization is the key to overcome these problems and to assure quality, safety and efficacy of medicinal drugs. Thin Layer Chromatography comes with priority in ensuring proper quality of plant materials; by its separation technique is very useful in quality evaluation of single drug and compound drug

preparations. In the present study, freshly collected unripened *Bilwa* fruits were selected and Thin Layer Chromatography was performed to obtain R_f values which were considered as standards which denotes the quality and purity of the drug. Market test samples of unripened *Bilwa* fruits were procured and Thin Layer Chromatography was performed to separate the compound mixture and to evaluate the quality by comparing the R_f values with that of genuine one. The study showed that R_f values of market test samples are similar to that of Standard sample, thus indicating that market samples of unripened *Bilwa* fruits are of genuine one.

Key words: Unripened fruit of *Bilwa*, Thin Layer Chromatography, R_f Value.

INTRODUCTION

Ayurveda the science of life is one of the oldest traditional systems of medicine and the origin of it can be traced to as early as dawn of the civilization and Vedic period. This system of medicine is based on the holistic approach and its doctrine encompasses the physical, chemical, biological and spiritual dimensions of life. Its aim is not just the cure of a disease but the maintenance of a positive healthy state of body, mind and spirit in a healthy environment and in harmony with the universe. In spite of the advancements in the modern medicine and discovery of effective drugs of synthetic origin in the recent past, the indigenous, traditional medicines still remain alive as precious cultural heritage in different societies of the world. These continue to cater in a significant manner to the medicinal needs of the third world countries which are inhabited by about 80 percent world's population. Moreover there has been renewed interest in the herbal medicines in present times and the apex body like WHO has recognized the potentialities of traditional systems of medicine in the management and self reliance of health care systems ^[1]. However, there are a lot of concerns about the traditional medicine in areas of quality, safety and efficacy.

Since ancient times, *Vaidyas* (Physicians) had identified medicinal plants, collected, processed, formulated and applied themselves. These plant drugs have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. In present era, due to rapid growth of population, forests have been destroyed and due to large demands there is scarcity of medicinal plants. Expectedly the number of plant species considered threatened in India has been progressively increasing. So Adulterants, Substitutes are common nowadays. As traders are the suppliers of raw materials, they are unaware of knowledge of medicinal plants and due to ignorance in garbling, storing, preserving etc. of drugs there is a wide variance in the quality of medicinal drugs. So the question arises about the safety and efficacy of drugs in present era. Hence, Standardization is the key to overcome these problems and to assure safety, efficacy and quality of medicinal drugs ^[2].

Even the World Health Assembly in resolutions has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards. Our own National Policy on Indian Systems of Medicine includes issues such as drug standards, regulations, enforcement and focuses the research agenda on drug standardization, pharmacology, toxicology, clinical trials ^[3].

Thin Layer Chromatography (TLC) is one of the standardization technique frequently used for evaluating medicinal plant materials and their preparation. Here study is performed for qualitative evaluation of market samples of unripened fruit of *Bilwa* by TLC.

MATERIALS AND METHODS

Five samples of unripened fruit of *Bilwa*.

- 1) A1 – Freshly collected sample from Nanded.
- 2) A2 – Market sample from Nanded.
- 3) A3 – Market sample from Bangalore.
- 4) A4 – Market sample from Jamnagar.
- 5) A5 – Market sample from Delhi.

All the five samples were made into coarse powder and stored in moisture free air tight container after suitable labeling. Preliminary Phytochemical analysis of samples was carried out for qualitative assessment of phytoconstituents as per the standard protocols.

Methanol extractives of samples were prepared by cold maceration.

Thin Layer Chromatography (TLC) is a separation technique used for the separation of compounds of mixture by their continuous distribution between two phases, one of which is moving past the other. It works on the basis of Adsorptive principle. The Retention factor (R_f) value of TLC is specific to solute and solvent system of that specific drug. This is characteristic chromatography pattern of every individual plant ^[5].

procedure - Extracts of the above sample drugs were prepared separately by taking 1gm of powder and 10 ml of methanol by cold maceration. Fine slurry of silica gel with binder Calcium Carbonate (stationary phase) was prepared in a beaker with distilled water. The slurry was then prepared on standard glass plates with the help of spreader and a thin layer was prepared about 250 μ m thickness. These plates were made to dry in air for 30 min, then in an oven at 110°C for another 30 min and allowed to cool, which makes the adsorbent layer active. By using micro capillaries the spots of the extract of above drugs not more than 4mm in diameter were placed on to the starting line of the plates, about 15 mm above the lower edge. Then the spots were allowed to dry. In the proportion of 4:4:2, Toluene:Ethylacetate:Methanol (Mobile phase) was taken. 10 ml of it was poured into separate chromatographic chambers. Then the chambers were closed and allowed to stand for at least 1hr at room temperature to achieve saturation of the chamber, in order to avoid unequal solvent evaporation losses from the developing plate. Then the plates were placed

into the chromatographic chambers ensuring that the spots were above the surface of the mobile phase. Then the chambers were closed and the solvents were allowed to flow by ascending type. The chromatograms were allowed to develop at room temperature ^[1].

After the mobile phase ascended to the specified distance the plates were removed from the chamber; solvent fronts were marked and allowed to dry at room temperature. The spots produced were observed under ultraviolet light. The color of each spot was noted and the center of each spot was marked with a needle. The distance from the center of each spot to the point of application was measured and recorded ^[4].

Then the R_f value of each spot was calculated by using the formula.

$$R_f = \frac{\text{Distance between the point of application and the center of the spot}}{\text{Distance between the point of application and the solvent front}}$$

Same procedure is carried out for another mobile phase of Toluene:Petroleum ether in the proportion of 9:1.

RESULTS AND DISCUSSION

Table 1: R_f values under 366nm uv light

Sr. No.	A1	A2	A3	A4	A5
1	0.38	0.38	0.38	0.38	0.38
2	0.48	0.48	0.48	0.48	0.48
3	0.59	0.59	0.59	0.59	0.59
4	0.62	0.62	0.62	0.62	0.62
5	0.76	0.79	0.76	0.79	0.79
6	0.79	0.86	0.79	0.86	0.86
7	0.86		0.86		

Mobile Phase –Toluene:Ethylacetate:Methanol (4:4:2)

As TLC is comparatively simple and rapid, the apparatus required is cheap and compound mixtures can be handled with comparative ease, hence is widely used in quality analysis of Ayurveda drugs. **standardization:** The R_f values of A1sample i.e., 0.38, 0.48, 0.59, 0.62, 0.79 and 0.86 in Toluene:Ethylacetate:Methanol mobile phase and R_f values 0.02, 0.03, 0.06, 0.11,0.12 in the mobile phase of Toluene:Petroleum ether are standard values to that drug. These standard values indicate the quality and purity of those drugs. It ensures the uniformity

of thought and practice so that each and everyone can understand it in the same manner.

Table 2: R_f values under 366nm uv light

Sr. No.	A1	A2	A3	A4	A5
1	0.02	0.02	0.02	0.02	0.02
2	0.03	0.03	0.03	0.03	0.03
3	0.06	0.06	0.06	0.06	0.06
4	0.11	0.11	0.11	0.11	0.11
5	0.12		0.17		

Mobile Phase –Toluene:Petroleum ether (9:1)

identification: TLC helps in proper identification of the right plant species. Standards are selected as a model to which other test samples can be compared. R_f values of A1 samples are standard values. Any other market test samples of unripened fruit of *Bilwa* can be compared with standard values. It should be similar to that of standard value, which indicates the genuine of that drug.

In this study, the R_f values of A2 to A5 market samples of *Bilwa* in Toluene: Ethylacetate: Methanol mobile phase are 0.38, 0.48, 0.59, 0.62, 0.79, 0.86 which are similar to standard values of A1 sample i.e., 0.38, 0.48, 0.59, 0.62, 0.79 and 0.86. Hence it indicates the market samples are of unripened fruit of *Bilwa* only. Then the R_f values of A2 to A5 market samples of *Bilwa* in the mobile phase of Toluene:Petroleum ether are 0.02, 0.03, 0.06, 0.11 which are similar to standard values of A1 sample i.e., 0.02, 0.03, 0.06 and 0.11. Hence it indicates the market samples are of unripened fruit of *Bilwa* only.

CONCLUSION

Hence it can be concluded that market samples of unripened fruit of *Bilwa* are of genuine one and Thin Layer Chromatography is a simple, cost effective technique useful in qualitative analysis of Ayurveda drugs.

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