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# Effect of Extraction Solvents on Phytochemicals of Whole Plant *Biophytum sensitivum* (L.) DC.

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**Abstract**

*Biophytumsensitivum* is an important medicinal plant consisting of various secondary metabolites. Considering the importance of *B. sensitivum* in medicinal purposes, this plant was selected for the present study. The extraction of phytochemicals from whole plant of *B. sensitivum* using different solvents such as Methanol, Hexane, Chloroform, Hydro- alcohol (Ethanol +Water) as well as the effect of extraction solvents on phytochemicals of whole plant *B. sensitivum*using Thin Layer Chromatography (TLC) were evaluated. It was found that Toluene: Ethyl acetate (8:2) was the best solvent system for the separation of chemical constituents in all the four different solvent extracts. This result was consistent in both cold and hot extraction method. The maximum and least band separation were observed in Chloroform extract and in Hydro-alcohol extract respectively. Based on the obtained experimental results we recommend Toluene: Ethyl acetate (8:2) in hot extract of Chloroform to be best for further studies to determine and compare the phytochemical activities of *B. sensitivum*.

## 1. Introduction

The plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. Traditional medicines still remain the main resource for a large majority of people treating health problems. Drugs from the plants are easily available, less expensive, safe, efficient and rarely have side effects. The medicinal plants represent an enormous reservoir of potential phytochemical compounds that could be useful as an alternative to allopathic drugs and are being used to develop Pharma drugs (Shanmugam *et al*., 2014).

The connection between human and their search for drugs in nature dates from the far past, of which there are enormous evidence from different sources (written documents, preserved monuments, and even original plant medicines). Awareness of medicinal plants usage is a result of the many years of struggles against diseases and man learned to pursue drugs in barks, seeds, fruits, and other parts of the plants. Contemporary science has acknowledged and considered in modern pharmacotherapy the active actions of plant origin drugs, known by ancient civilizations and used throughout the millennia (Srivastava, 2018).

In recent times, research on plants has increased across the globe owing to their immense potential to heal life-threatening diseases. A

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number of medicinal plants have been evaluated for their therapeutic potentials; most of them have shown their protective effects against various diseases. The secondary metabolites, especially the bioactive compounds present in the plants, provided the basis for several sophisticated traditional medicine systems like Ayurveda, Unani, Folk, and Chinese (Ameenah, 2006).

During the last few decades, extensive research has been carried out to elucidate the chemistry, biological activities, and medicinal applications of *B. sensitivum* (Sakthivel & Guruvayoorappan, 2012). *Biophytum sensitivum* (L.) DC. Belonging to Division: Magnoliophyta, Class: Magnoliopsida, Order: Oxalidales, Family: Oxalidaceae, found in wet lands of tropical India, South Asia and Africa. Normally, it is present in the shades of trees and shrubs, in grass lands at low and medium altitudes. It is commonly known as Life plant (English). In Kerala, It is commonly known as Mukkutti (Jirovetz *et al.*, 2004; Inngjerdingenet *al.*, 2006). The flower of this plant is considered as one of the ten sacred plants which are called as Dasapushpam in tradition and culture of Kerala state in India (Varghese *et al.*,2010). The Mukkutti (flowers) are significant for the people of Kerala, for its medicinal, cultural and traditional values. *B. sensitivum* is used as a traditional folk medicine in ailments such as inflammation, arthritis, wounds, tumors and burns, gonorrhea, stomach ache, asthma, cough, degenerative joint disease, urinary calculi, diabetes, snake bite, amenorrhea and dysmenorrheal (Bharati & Sahu, 2012). Generally, the whole plant is frequently used for medicinal purpose. But an ethnopharmacological survey of six medicinal plants in Mali and West Africa showed that most of the traditional preparations are made from leaves (Gronhaug *et al.,* 2008).

Solvent extraction is most frequently used technique for isolation of plant metabolites. However, the extract yields of the plant materials strongly depend on the nature of extracting solvent, due to the different solubility of the chemical compounds present in it. Identification of most effective extraction solvents to increase the yield of raw materials is of great importance as it helps reducing the quantity required for medicine manufacture. *B. sensitivum* was chosen for the current study because of its significance in medicinal applications. The extraction of phytochemicals from whole plant of *B. sensitivum* using different solvents such as Methanol, Hexane, Chloroform, Hydro- alcohol (Ethanol +Water) as well as the effect of extraction solvents on phytochemicals of whole plant *B. sensitivum* using Thin Layer Chromatography (TLC) were also evaluated.

## 2. Methodology

### 2.1. Collection, identification and drying of Plant Material

The plant used for the investigation *B. sensitivum* was collected in fresh condition from natural habitats of Kozhikode district and was identified and authenticated at Department of Botany, St. Joseph's College, Devagiri. Voucher specimen of the same was deposited at DEV herbarium for further reference.

The plant material was washed thoroughly with water. The leaves, stem and roots were cut into small pieces and were shade dried until the chopped parts became dried for grinding. After drying, the plant materials were ground separately using mechanical blender into fine powder and transferred into airtight containers at ambient temperature with proper labelling for future use.

**2.2. Preparation of extracts**

**2.2.1 Hot extracts**

The extracts of the *B.sensitivum* were prepared using different solvents such as Hexane, Chloroform, Methanol, Hydro-alcohol (Ethanol + Water). The extraction was done using Reflex condenser. Dried and powdered plants were taken in the RB flask containing methanol (200ml). The setup at boiling temperature was kept for about four hours. The extracts thus obtained were filtered and concentrated to 5ml in a water bath. Reflex condenser is a distillation technique involving the condensation of vapours and the return of this condensate to the system from which it originated. It is used in industrial and laboratory distillations. It is also used in chemistry to supply energy to reactions over a long period of time. The term reflux is very widely used in industries that utilize large scale distillation column and chemical plants and natural gas possessing plants. A liquid reaction mixture is placed in a vessel open at the top. This vessel is connected to a condenser, such that any vapours given off are cooled back to the liquid and fall back into the reaction vessel. The vessel is then heated vigorously for the course of the reaction. The purpose is to thermally accelerate the reaction by conducting it at an elevated temperature (*i.e.*, the solvents boiling point). The advantage of this technique is that it can be left for a long period of time without the need to add more solvent or fear of the reaction vessel boiling dry as any vapour is immediately condensed in the condenser **(Table-1).**

**2.2.2 Cold extracts**

The dried *B.sensitivum* whole plant powder then further used for extraction purpose for cold method, using various solvents{Hexane, Chloroform, Methanol, Hydro-alcohol (Ethanol + Water)}. Weigh the powder and added into conical flask with respective solvents and allow keeping at room temperature for thirty-minute shaking after each twenty-four hours for seven days. The extract is separated on flexible (Aluminium-backed) Silica TLC plates, and bands are visualized under ultraviolet (UV) light.

The developed bands in the TLC plates were analysed by its retention factor (Rf), which were determined for various examples utilizing the accompanying formula [Gujjeti & Mamidala, 2013].

Rf = (Distance travelled by the solute)/ (Distance travelled by the solvent front on TLC plates) **(Table-2).**

**2.3 Chemicals and reagents used in the study**

Solvents such as Methanol, Ethanol, Chloroform and Hexane purchased from Merck and HiMedia were used. All other chemicals employed were of standard analytical grade from Merck, India.

## 3. Results & Discussion

The TLC profiles were developed for whole plant*B. sensitivum* with various solvents like Methanol, Hexane, Chloroform and Hydro-alcohol (Ethanol+Water). Five different solvent systems were used to separate the phytochemicals. These were Toluene: Ethyl acetate (9:1), Toluene: Ethyl acetate (8:2), Toluene: Ethyl acetate: Methanol (7:3:1), Toluene: Ethyl acetate: Methanol (7:2:1), Toluene: Ethyl acetate: Methanol: Acetic acid (7:3:1:0.1).

Toluene: Ethyl acetate (8:2) was the best solvent system for the separation of chemical constituents in all the four different solvent extracts. Differences were observed in the number of bands of the TLC profiles developed for whole plant *B. sensitivum*with various solvents like

Methanol, Hexane, Chloroform and Hydro-alcohol (Ethanol+Water).

### 3.1. TLC profiling

#### 3.1.1 The solvent system Toluene: Ethyl acetate (9:1)

In hot extraction method resulted seven different bands were separated in Methanol extract, eight bands were separated in Hexane extract, eight bands were formed in Chloroform extract. However, none were observed in Hydro-alcohol. Similarly, in cold extract seven bands were separated in Methanol extract, seven bands were separated in Hexane extract, twelve bands were separated in Chloroform extract and there were no bands observed in Hydro –alcohol. The maximum band separations werefound in Chloroform extract and the least in Hydro –alcohol. In hot extraction method same Rf value 0.62 and 0.68 were found in all the extract except Hydro- alcohol. Similarly, the Rf value 0.15 were also found in both Methanol and Chloroform. The cold extract also resulted same Rf value 0.18 in all the solvent extract except Hydro-alcohol.

#### 3.1.2 The solvent system Toluene: Ethyl acetate (8:2)

In hot extraction, seventeen bands were separated in Methanol extract, fourteen bands were separated in Hexane extract, thirteen bands were separated in Chloroform extract and four bands were separated in Hydro –alcohol. However, in cold extraction eight bands were observed in Methanol extract, six bands were observed in Hexane, eleven bands were observed in Chloroform and no bands were observed in Hydro-alcohol. The maximum band separation observed in Methanol extract in hot extraction method likewise chloroform in cold extraction method. Here also the least number of bands were found in Hydro-alcohol. The same Rf value 0.86 were observed in the cold extraction of Methanol, Hexane and Chloroform. In hot extraction method Rf value 0.67 found in Methanol and Chloroform extract.

#### 3.1.3 The solvent system Toluene: Ethyl acetate: Methanol (7:3:1)

The hot extraction of Methanol resulted ten bands, Hexane resulted eleven bands, Chloroform resulted twelve bands, only one band was separated in Hydro- alcohol. The cold extraction of Methanol resulted ten bands, Hexane resulted seven bands, Chloroform resulted twelve bands and none of the bands were resulted in Hydro-alcohol. The maximum number

of separation observed in Chloroform extract and minimum number of band separation observed in Hydro-alcohol in both Hot and cold extract method. Likewise, Same Rf value 0.51 was observed in hot extract of Methanol and Hexane. However, Rf value 0.71 and 0.83 were observed in cold extract of Methanol and Chloroform.

**3.1.4 The solvent system Toluene: Ethyl acetate: Methanol (7:2:1)**

The hot extraction of Methanol resulted eight bands, Hexane resulted nine bands, Chloroform resulted eleven bands and none of the bands were found in Hydro- alcohol. The cold extraction of Methanol resulted six bands, Hexane resulted five bands, Chloroform resulted thirteen bands and no bands were found in Hydro-alcohol. Here the maximum band separation were resulted the Chloroform extract and the least number of bands in Hydro-alcohol. The cold extract of Methanol and Chloroform resulted the same Rf value 0.86 and in hot extract of Methanol and Hexane resulted the same Rf values 0.75, 0.93 and 0.96. Similarly, Rf value 0.11 observed in Hexane and Chloroform.

**3.1.5. The solvent system Toluene: Ethyl acetate: Methanol: Acetic acid (7:3:1:0.1)**

The hot extraction of Methanol resulted seven bands, Hexane extract resulted seven bands, Chloroform extract resulted seven bands and Hydro-alcohol resulted two bands. In cold extract method Methanol resulted eight bands, Hexane resulted three bands, Chloroform resulted nine bands and no bands were separated in Hydro- alcohol.In hot extract of Methanol, Hexane and Chloroform resulted same number of bands and the Hydro- alcohol resulted the least number of bands. Likewise, Rf value 1 were observed in all solvent extract except Hydro- alcohol and Rf value 0.68 were found in both Methanol and Hexane. The cold extract of Methanol and Hexane resulted the same Rf values 0.91 and 0.98.

**Table 1: Phytochemical test results of *B. sensitivum*- Hot extraction**

| No. | Extract name | | Solvent system I | Solvent systemII | Solvent system III | Solvent system IV | Solvent system V |
|---|---|---|---|---|---|---|---|
| | | | ( 9:1), | (8:2) | (7:3:1) | (7:2:1) | (7:3:1:0.1) |
| 1. | Methanol | No of bands | 7 | 17 | 10 | 8 | 7 |
| | | Rf Value | 0.07 | 0.04 | 0.25 | 0.22 | 0.82 |
| | | | 0.15 | 0.06 | 0.38 | 0.26 | 0.56 |
| | | | 0.33 | 0.09 | 0.43 | 0.46 | 0.63 |
| | | | 0.4 | 0.11 | 0.48 | 0.75 | 0.68 |
| | | | 0.55 | 0.16 | 0.51 | 0.82 | 0.86 |
| | | | 0.62 | 0.21 | 0.53 | 0.93 | 0.7 |
| | | | 0.68 | 0.25 | 0.75 | 0.96 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0.28 | 0.78 | 0.98 | |
| | | | | 0.3 | 0.83 | | |
| | | | | 0.58 | 0.88 | | |
| | | | | 0.64 | | | |
| | | | | 0.67 | | | |
| | | | | 0.75 | | | |
| | | | | 0.83 | | | |
| | | | | 0.91 | | | |
| | | | | 0.95 | | | |
| | | | | 1 | | | |
| **2.** | Hexane | No of bands | 8 | 14 | 11 | 9 | 7 |
| | | Rf Value | 0.06 | 0.01 | 0.23 | 0.11 | 0.51 |
| | | | 0.08 | 0.03 | 0.37 | 0.23 | 0.62 |
| | | | 0.13 | 0.17 | 0.42 | 0.27 | 0.68 |
| | | | 0.35 | 0.21 | 0.46 | 0.4 | 0.83 |
| | | | 0.42 | 0.26 | 0.51 | 0.47 | 0.93 |
| | | | 0.53 | 0.4 | 0.65 | 0.68 | |
| | | | 0.62 | 0.58 | 0.73 | 0.75 | 0.73 |
| | | | 0.68 | 0.63 | 0.77 | 0.93 | 1 |
| | | | | 0.7 | 0.85 | 0.96 | |
| | | | | 0.78 | 0.87 | | |
| | | | | 0.86 | | | |
| | | | | 0.88 | | | |
| | | | | 0.97 | | | |
| | | | | 1 | | | |
| **3.** | Chloroform | No of bands | 8 | 13 | 12 | 11 | 7 |
| | | Rf Value | 0.06 | 0.01 | 0.23 | 0.11 | 0.47 |
| | | | 0.1 | 0.05 | 0.3 | 0.18 | 0.55 |
| | | | 0.15 | 0.07 | 0.37 | 0.25 | 0.61 |
| | | | 0.37 | 0.1 | 0.42 | 0.27 | 0.66 |
| | | | 0.43 | 0.16 | 0.47 | 0.41 | 0.85 |
| | | | 0.53 | 0.26 | 0.5 | 0.5 | 0.96 |
| | | | 0.62 | 0.57 | 0.57 | 0.72 | 1 |
| | | | 0.68 | 0.62 | 0.62 | 0.76 | |
| | | | | 0.67 | 0.72 | 0.82 | |
| | | | | 0.76 | 0.76 | 0.92 | |
| | | | | 0.85 | 0.82 | 0.97 | |
| | | | | 0.92 | 0.86 | | |
| | | | | 1 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **4.** | Hydro-alcohol (Ethanol+Water) | No of bands | 0 | 4 | 1 | 0 | 2 |
| | | Rf Value | | 0.24 | 0.38 | | 0.43 |
| | | | | 0.61 | | | 0.58 |
| | | | | 0.81 | | | |
| | | | | 0.87 | | | |

**Table 2: Phytochemical test results of *B. sensitivum*- Cold extraction**

| **No.** | **Extract name** | | **Solvent system I** | **Solvent systemII** | **Solvent system III** | **Solvent system IV** | **Solvent system V** |
|---|---|---|---|---|---|---|---|
| | | | **( 9:1),** | **(8:2)** | **(7:3:1)** | **(7:2:1)** | **(7:3:1:0.1)** |
| **1.** | Methanol | No of bands | 7 | 8 | 10 | 6 | 8 |
| | | Rf Value | 0.04 | 0.13 | 0.15 | 0.13 | 0.15 |
| | | | 0.18 | 0.26 | 0.3 | 0.18 | 0.33 |
| | | | 0.32 | 0.42 | 0.4 | 0.27 | 0.5 |
| | | | 0.33 | 0.5 | 0.48 | 0.33 | 0.57 |
| | | | 0.36 | 0.6 | 0.58 | 0.86 | 0.63 |
| | | | 0.52 | 0.75 | 0.67 | 0.97 | 0.75 |
| | | | 0.75 | 0.86 | 0.76 | | 0.91 |
| | | | | 0.97 | 0.8 | | 0.98 |
| | | | | | 0.9 | | |
| | | | | | 0.93 | | |
| **2.** | Hexane | No of bands | 7 | 6 | 7 | 5 | 3 |
| | | Rf Value | 0.02 | 0.36 | 0.32 | 0.21 | 0.91 |
| | | | 0.1 | 0.58 | 0.47 | 0.31 | 0.96 |
| | | | 0.18 | 0.63 | 0.71 | 0.78 | 0.98 |
| | | | 0.38 | 0.82 | 0.8 | 0.96 | |
| | | | 0.46 | 0.86 | 0.83 | 1 | |
| | | | 0.67 | 1 | 0.92 | | |
| | | | 0.86 | | 0.95 | | |
| | | | | | | | |
| **3.** | Chloroform | No of bands | 12 | 11 | 12 | 13 | 9 |
| | | Rf Value | 0.08 | 0.04 | 0.32 | 0.16 | 0.47 |
| | | | 0.1 | 0.06 | 0.41 | 0.25 | 0.52 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0.15 | 0.21 | 0.47 | 0.31 | 0.58 |
| | | | 0.18 | 0.35 | 0.52 | 0.33 | 0.63 |
| | | | 0.28 | 0.46 | 0.56 | 0.48 | 0.83 |
| | | | 0.37 | 0.58 | 0.6 | 0.53 | 0.9 |
| | | | 0.43 | 0.65 | 0.63 | 0.67 | 0.96 |
| | | | 0.55 | 0.73 | 0.71 | 0.77 | 0.98 |
| | | | 0.6 | 0.82 | 0.8 | 0.85 | 1 |
| | | | 0.66 | 0.86 | 0.83 | 0.91 | |
| | | | 0.72 | 0.98 | 0.9 | 0.93 | |
| | | | 0.85 | | 0.92 | 0.97 | |
| | | | | | | 1 | |
| **4.** | Hydro-alcohol (Ethanol+Water) | No of bands | 0 | 0 | | 0 | 0 |
| | | Rf Value | | | | | |

**4. Conclusion**

In the present study the effect of extraction solvents on phytochemicals of whole plant*B. sensitivum* have been investigated using Thin layer chromatographic technique. Toluene: Ethyl acetate (8:2) was the best solvent system for the separation of chemical constituents in all the four different solvent extracts. This result was consistent in both cold and hot extraction method. Disparities were observed in terms of number of bands and band intensity of the TLC profiles developed for whole plant *B. sensitivum* with various solvents like Methanol, Hexane, Chloroform and Hydro-alcohol which specify the qualitative and quantitative deviation in chemical constituents. The maximum and least band separation were observed in Chloroform extract and in Hydro- alcohol extract respectively.Based on the obtained experimental results we recommend that, Toluene: Ethyl acetate (8:2) in hot extract of Chloroform to be best for further studies to determine and compare the phytochemical activities of *B. sensitivum*.

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