

**RESEARCH ARTICLE****PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHIC STUDIES OF *ANNONA SQUAMOSA* (SEEDS), *AZADIRACHTA INDICA* (LEAVES) AND *LAVANDULA ANGUSTIFOLIA* (FLOWER) AQUEOUS EXTRACT**

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[purva.tiwari29@gmail.com](mailto:purva.tiwari29@gmail.com)**Abstract**

India being a rich and varied flora of medicinal plants since the Vedic period. The present study deals with the phytochemical screening and thin layer chromatographic studies of *Annona squamosa*, *Azadirachta indica* and *Lavandula angustifolia* aqueous extract belonging to family Annonaceae, Meliaceae and Lamiaceae respectively. Phytochemical screening determination by some chemical tests and thin layer chromatographic study was carried out by using various solvent system of varying polarity of hexane, chloroform, ethyl acetate, acetone and methanol extracts. Phytochemical screening reflects presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavanoids, quinones and terpenoids shows different types of results in different plant extracts. Thin layer chromatographic studies of the *Annona squamosa*, *Azadirachta indica* and *Lavandula angustifolia* aqueous extracts constituted different colored phytochemical compounds with different Rf values. The aqueous extracts in the drug is carried out to establish the biomarker compound. The result obtained in present study indicated *Annona squamosa*, *Azadirachta indica* and *Lavandula angustifolia* as a rich source of natural antioxidants and provides evidence that aqueous extract of *Annona squamosa*, *Azadirachta indica* and *Lavandula angustifolia* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

**Keywords:** *Annona squamosa*, *Azadirachta indica*, *Lavandula angustifolia*, Phytochemical, Retention factor, TLC Studies. Phytochemical, Chromatography.

**INTRODUCTION**

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. Medicinal plants are great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes as reported by Okwu, D. E. and Hill A.F.<sup>1-3</sup> Herbs being easily available to human beings have been explored to the maximum for their medicinal properties.

*Annona squamosa* L., the plant leaves of Annonaceae family, are used as insecticidal and antispasmodic agents and are used in the treatment of rheumatism and painful spleen. The plant is reported to possess analgesic, anti-inflammatory, antipyretic, antiulcer, and antiseptic and abortifacient activities. Its use as an insecticidal agent has been investigated by several workers and various phytochemical, pharmacological, antibacterial and antiovolatory studies have already been carried out with the seed

extracts.<sup>4</sup> *Azadirachta indica*, commonly known as Neem, belongs to Family Meliaceae, is one of the most versatile medicinal plants that has gained worldwide importance due to medicinal and insecticide properties. There are several studies showing the effects of *Azadirachta indica* in experimental and clinical models.<sup>5</sup> *Lavandula angustifolia* (lavender or English lavender though not native to England; also garden lavender, common lavender, true lavender, narrow-leaved lavender), formerly *L. officinalis*, is a flowering plant in the family Lamiaceae, native to the western Mediterranean, primarily the Pyrenees and other mountains in northern Spain.<sup>6</sup>

The aim of this research study was to assess the bioactive components present in the aqueous extract of *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) using phytochemical screening and chromatographic analysis.

## **MATERIALS AND METHODS**

### **Collection of plant**

The plant *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) were collected from local area of Bhopal (M.P.) in the month of Oct - Nov., 2016.

### **Preparation of plant extract**

Drying of fresh plant parts was carried out in sun but under the shade. Dried *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) were preserved in plastic bags and closed tightly and powdered as per the requirements.

### **Extraction**

Following procedure was adopted<sup>7</sup> for the preparation of aqueous extracts from the shade dried and powdered herbs:

### **Defatting of plant material**

Powdered material of *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80 °C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

### **Extraction by hot continuous Soxhletion process**

Dried powdered *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) has been extracted with aqueous (Water) using Soxhlet's apparatus for 48 hrs, filtered and dried using vacuum evaporator at 40 °C.

### **Determination of percentage yield**

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

### Phytochemical Screening

Chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, flavonoids, and tannins, in the medicinal plants under study were carried out in extracts by using standard procedure in.<sup>8,9</sup>

### Thin layer chromatographic studies

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Hexane: Acetic acid (9:1) solvent system I, In solvent system II Hexane: Ethyl acetate :Acetic acid (5:4:1), In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1) used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor ( $R_f$ ), values were calculated for different samples.<sup>10</sup>

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

## RESULTS AND DISCUSSION

The crude extracts so obtained after the soxhlet extraction process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from different samples using methanol as solvents are depicted in the table 1.

**Table 1: Percentage yield of plant material**

S. No.	Extract	% Yield (w/w)
1	<i>Annona squamosa</i>	3.56%
2	<i>Azadirachta indica</i>	4.25%
3	<i>Lavandula angustifolia</i>	9.85%

The yield of aqueous extracts is shown in Table 1. The amount obtained from *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) methanolic extracts are 3.56%, 4.25% and 9.85% (w/w) respectively.

A small portion of the dried extracts were subjected to the phytochemical analysis to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table.

**Table 2: Result of phytochemical screening**

Chemical Tests	<i>Annona squamosa</i>	<i>Azadirachta indica</i>	<i>Lavandula angustifolia</i>
<b>Alkaloids</b>			
<i>Mayer's reagent</i>	-	-	+
<i>Hager's reagent</i>	-	-	+
<i>Wagner's reagent</i>	-	-	-
<i>Dragendorff's reagent</i>	-	-	+
<b>Glycosides</b>			
<i>Baljet test</i>	+	+	-
<i>Legal's test</i>	+	+	-
<i>Keller-Kiliani</i>	+	+	-

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<b>Phenols/Tannins</b>			
<i>Ferric chloride</i>	+	+	+
<i>Gelatin Solution</i>	+	+	+
<i>Lead acetate test</i>	-	-	+
<b>Flavonoids</b>			
<i>FeCl<sub>3</sub> test</i>	-	+	-
<i>Alkaline reagent test</i>	+	-	+
<i>Shinoda test</i>	+	+	+
<b>Saponins</b>			
<i>Foam test</i>	+	+	+
<i>Hemolytic test</i>	+	+	-
<i>Lead acetate</i>	-	-	+
<b>Fixed oil/Fats</b>			
<i>Spot</i>	-	-	-
<i>Saponification</i>	-	-	-
<b>Gums &amp; Mucilage</b>			
Water	-	-	-
<b>Carbohydrates</b>			
<i>Molish test</i>	-	-	-
<i>Fehling's solution test</i>	-	-	-
<i>Benedict's test</i>	-	-	-
<b>Amino acids</b>			
<i>Ninhydrin Test</i>	-	-	-
<i>Millons Test</i>	-	-	-
<i>Xantoprotein Test</i>	-	-	-

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<b>Terpenoids</b>			
<i>Lieberman Burchard Test</i>	+	+	+
<i>Salkowski test</i>	+	+	+
<b>Steroids</b>			
<i>Lieberman Test</i>	-	+	-
<b>Protein</b>			
<i>Biuret test</i>	-	-	-

+ indicates present; - indicates absent

The present study carried out in the *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) aqueous extract revealed the presence of various medicinal active constituents. The phytochemical active compounds aqueous extract of *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) were qualitatively analysed and the results are presented in Table 2.

**Table 3: Calculation of R<sub>f</sub> Value**

S. No.	Compound	Extract	R <sub>f</sub> Value
1.	Gallic acid	Toluene: Ethyl acetate: Formic acid (7:5:1)	0.65
2.	Quercetin	Toluene: Ethyl acetate: Formic acid (5:4:1)	0.82
3.	<i>Annona squamosa</i>	Toluene: Ethyl acetate: Formic acid (5:4:1)	0.54, 0.82
4.	<i>Azadirachta indica</i>	Toluene: Ethyl acetate: Formic acid (5:4:1)	0.38, 0.82
5.	<i>Lavandula angustifolia</i>	Toluene: Ethyl acetate: Formic acid (7:5:1)	0.65, 0.44, 0.76

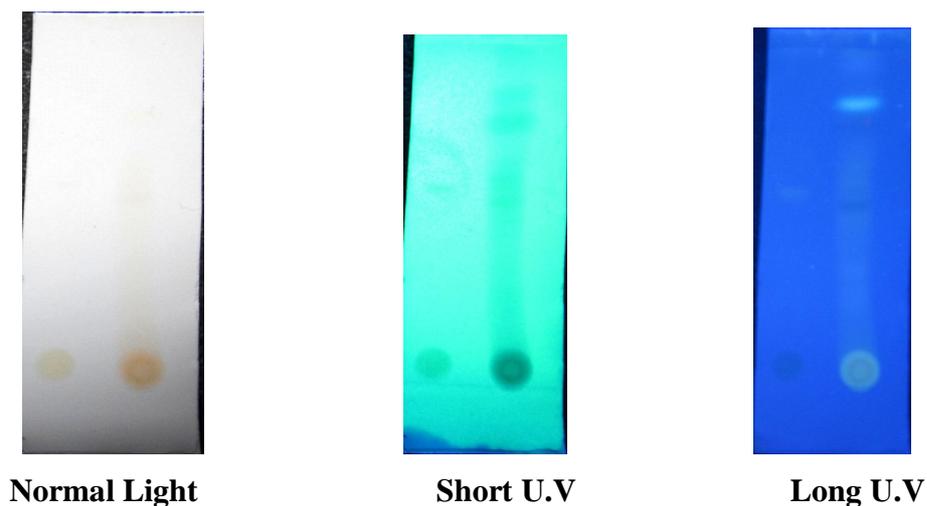


Figure 1: Photograph of T.L.C (*Azadirachta indica*)

Spot-1 Standard, Spot-2 Extract

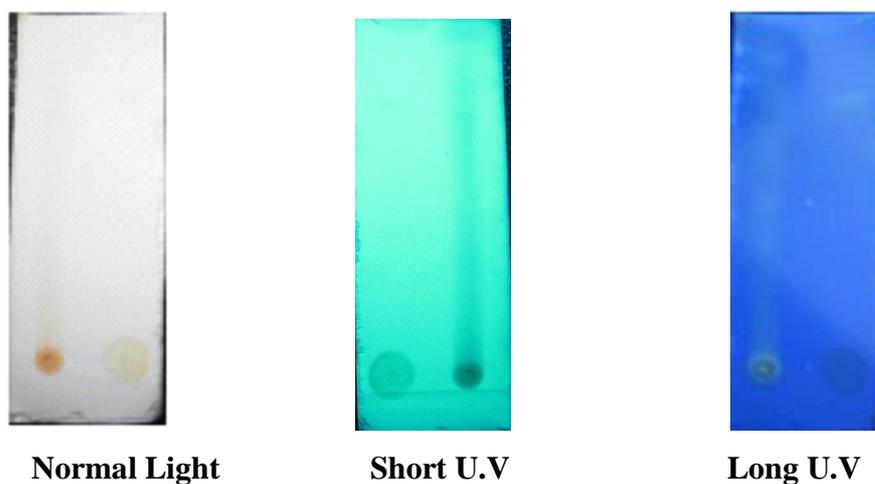


Fig. 2: Photograph of T.L.C (*Annona squamosa*)

Spot-1 Standard, Spot-2 Extract

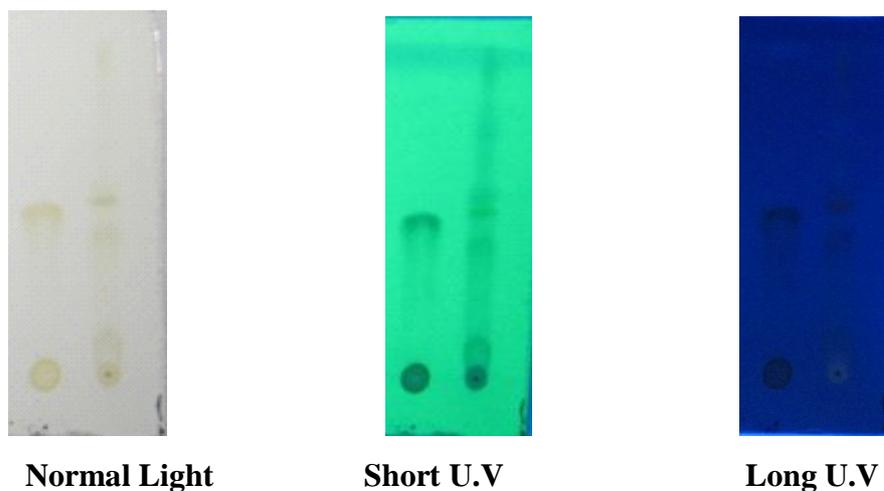


Fig. 3: Photograph of T.L.C (*Lavandula angustifolia*)

Spot-1 Standard, Spot-2 Extract

A large number of solvent systems were tried to achieve a good resolution. Finally, the solvents Toluene: Ethyl acetate: Formic acid (7:5:1) and Toluene: Ethyl acetate: Formic acid (5:4:1) was used. Results of thin layer chromatographic studies of the aqueous extract of *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) are tabulated in table 3.

A large number of plants produce secondary metabolites such as alkaloids, flavanoids, phenols, terpenoids, steroids and quinines that are used in pharmaceuticals, cosmetics and pesticide industries. Thus the present study confirms the traditional medical practice and previous pharmacological observations and supplement treatment for other health problems such as allergic reactions, arthritis, some malignancies, and diseases resulting from hormone deficiencies or abnormal production etc: in.<sup>8,9</sup> In the present study, phytochemical screening for all three extracts showed significant indication about the presence of metabolites. Alkaloids, saponinis, tannins, amino acids, flavonoids and terpenoids were found to be present in the aqueous extract of *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower). The results of the present study also supplement the folkloric usage of the studied plants which possess several known and unknown bioactive compounds with bio-activity. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders.

TLC profiling of all three extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R<sub>f</sub> values in different solvent system. This variation in R<sub>f</sub> values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the R<sub>f</sub> values of compounds in different solvent system. Different R<sub>f</sub> values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

### **Conclusion**

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. The phytochemical screening and chromatographic analysis of aqueous extract of *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) have shown the presence of pharmacologically active substances such as alkaloids, steroids and flavoniods. Further purification, identification and characterization of the active compounds would be our priority in future studies.

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