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Phytochemical screening and thin layer Chromatography of medicinal plant *Azima tetracantha*. L

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Abstract
Medicinal plants have bioactive secondary component, which are used to control of several diseases. Medicinal plant *Azima tetracantha* which possess own pharmaceutical activities and are applied directly to snake bite, stomach disorders, toothache, bleeding gums, rheumatism and chronic diarrhea. In the present investigation the phytochemical studies were carried out in *Azima tetracantha* leaves and to detect the presence of secondary metabolites like alkaloids, steroids, flavonoids, terpenoids, saponins, tannins, phenols, coumarins and glycosides. The solvent system selected for the results of TLC was 100 ml petroleum ether, 11ml acetone and 1ml distilled water and identified spot in the aqueous extract. The Rf values of the extract was 0.2. The study will provide the presence of secondary metabolites for the leaf extract of *Azima tetracantha* to exhibit therapeutic properties.

Keywords: *Azima tetracantha*, phytochemical studies, extraction, TLC

Introduction
Medicinal plants are relied upon by 80% of world's population and in India, the use of medicinal plants as therapeutic agents remains an important component of the traditional system [1]. The medicinal plants are the best source to obtain a variety of new herbal drugs reported by World Health Organization. Three fourth of the individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Hence, such plants should be recognize their properties, safety and efficacy. The assistance of plant extract and phytochemicals with antimicrobial properties can be a great significance in therapeutic treatments. In the past years, to prove such efficiency a number of studies have been conducted in different countries. Most of the plant extracts have been used for antimicrobial traits, which are synthesized during secondary metabolism of the plant [2]. Medicinal plants for evaluation of antioxidant phytochemicals such as phenols, flavonoids and tannins which have received more attention for their potential role in prevention of human diseases [3]. Chromatographic and spectral fingerprint analysis plays an important role in the quality control of complex herbal medicines [4]. Thin layer chromatography (TLC) is used to identify the phytochemical constituents in a sample [5].
Azima tetracantha belongs to the family Salvadoraceae, commonly known as "mulluchangu". It happens naturally in the central, eastern and South Africa as well as in Indian Ocean islands and extends through Arabia to tropical Asia [6]. In Madagascar, the infusion of the leaves is used to treat venereal diseases. In India and Sri Lanka the root bark and leaves are used as a remedy for rheumatism.

Materials and Methods
Collection of plant materials
The medicinal plant *A. tetracantha* free from diseases were collected from in and around Srivilliputhur and Krishnankoil, Virudhunagar District, Tamil Nadu, India (It is located 9.51 latitude and 77.63 longitude) (fig.1). The plant parts were removed, washed thoroughly with running tap water and again washed with sterile distilled water to remove dirt prior to drying process. The leaves of *A. tetracantha* were shade dried at room temperature for a week to remove the moisture content and powdered by using mixer grinder [7].

Fig 1: *Azima tetracantha*

Preparation of Leaf extract
About 10 g of these finely powered leaves of *A. tetracantha* was weighed separately and dissolved into 100 ml distilled water and boiled for about 20 min. Then the extracts were filtered thrice through Whatman No. 1 filter paper (HiMedia) to remove particulate matter and to get clear

solutions which were then refrigerated (4°C) in 250 ml Erlenmeyer flasks for further experiments (fig.2) [7].

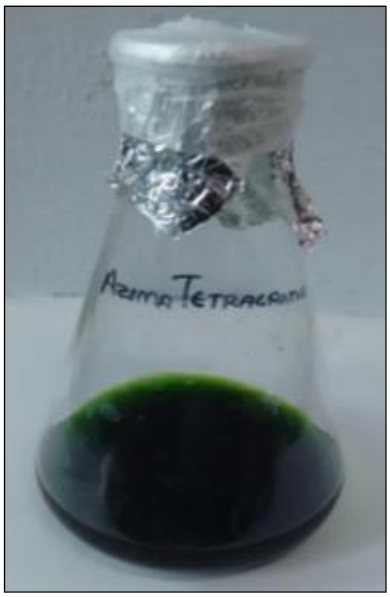

Fig 2: Leaf extract of *Azima tetracantha*

Phytochemical analysis
One hundred gram of powdered leaf sample was extracted with hexane, acetone, methanol and aqueous by Soxhlet apparatus. Phytochemical analysis was carried out with the following methods.

Test for carbohydrate
1ml of solvents and aqueous leaf extract of *A. tetracantha* was taken in the test tube and added 1ml of Benedict's reagent. The mixture was kept in the water bath for 5 minutes. Formation of orange red color indicates the presence of a high amount of carbohydrate and the light green indicates the traceable amount of carbohydrates present in the plant [8].

Test for alkaloids
1ml of solvents and aqueous leaf extract of *A. tetracantha* was taken in the test tube and added 1ml of Mayer's reagent. In the bottom of the test tube, precipitate formed indicates the presence of alkaloids [9].

Test for Steroids
1ml of solvents and aqueous leaf extract of *A. tetracantha* was individually dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns yellow and sulphuric acid layer showed green fluorescence. This indicates the presence of steroids [10].

Test for flavonoids
2 ml of solvents and aqueous leaf extract of *A. tetracantha* was mixed with 2 ml of 2% solution of NaOH. The formation of yellow colour which turned colourless on addition of few drops of diluted acids which indicate the presence of flavonoid [11].

Test for terpenoids
5 ml of solvents and aqueous leaf extract of *A. tetracantha* was mixed with 2ml of chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colour was formed to show positive result for the presence of terpenoid [12].

Test for Saponins
5 ml of solvents and aqueous leaf extract of *A. tetracantha* was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins [13].

Test for Tannins
2 ml of solvents and aqueous leaf extract of *A. tetracantha* added with a few drops of 1% lead acetate. A yellowish precipitate indicates the presence of tannins [14].

Test for phenols
2 ml of solvents and aqueous leaf extract of *A. tetracantha* was mixed with 2 ml of 2% $FeCl_3$ solution. A blue green colour or black coloration indicates the presence of phenols [15].

Test for Leucoanthocyanins
5 ml of solvents and aqueous leaf extract of *A. tetracantha* was added to 5 ml of isoamyl alcohol. Upper layer appears red in colour indicates the presence of leucoanthocyanins [16].

Test for Coumarins
3 ml of 10% NaOH was added to 2 ml of solvents and aqueous leaf extract of *A. tetracantha*. The yellow colour formation indicates the presence of coumarins [17].

Test for Glycosides
To a small portion solvents and aqueous leaf extract of *A. tetracantha* was added with 2ml of glacial acetic acid and one drop of ferric chloride was added followed by 1ml of conc. sulphuric acid and it was mixed well. Appearance of brown color indicates the presence of glycosides [18].

Thin Layer Chromatography (TLC)
Aqueous extract of *Azima tetracantha* sample was subjected to TLC studies. For the TLC analysis, the dimensional ascending method was used [19]. 20×20 cm glass TLC plate coated with silica gel 60G F254, was cut with a scissor in 14×3 cm shape. TLC plate was marked with the pencil softly 1.5 cm far from the both bottom and top. Glass capillaries were used to spot the sample on the TLC plate on the pencil marked bottom line. Then it was placed to dry the plate and loaded the sample again until a dark spot is obtained. The solvent contains 100 ml petroleum ether, 11ml acetone and 1ml distilled water (modified) about 20ml was taken in the chamber. The plate was placed in the chamber lining on the top. After the run, plates were dried and then used to detect the spots. The movement of the active compound was expressed by the retention factor (Rf) [20].

Rf = Distance moved by the solute / Distance moved by the solvent.

Results and Discussion
The results for phytochemical analysis were tabulated in (Table 1). Each of the extracts differs with phytochemicals alkaloids, steroids, flavonoids, terpenoids, saponins, tannins, phenols, coumarins and glycosides. The hexane extract revealed the presence of secondary metabolites such as steroids, flavonoids, tannins, phenols and coumarins. The

acetone extract obtained with alkaloids, flavonoids, saponins and coumarins. The methanol extract contained steroids, flavonoids, tannins, phenols, coumarins and glycosides. The aqueous extract obtained flavonoids, terpenoids, saponins, tannins, phenols, coumarins and glycosides. These detections were good enough to reflect its importance. The preliminary phytochemical screening was carried out from *Ajuga remota* extracts. The presence of flavonoids, tannins, saponins, phenolic compounds and steroids [21]. Similarly *Justicia adhatoda* leaf extract showed the presence of alkaloid, flavonoids, glycosides, tannins, protein, resins and phenol in the methanol, chloroform, ethyl acetate and diethyl ether [22]. Similarly phytochemical screening of aqueous methanol fraction of *Hibiscus asper* leaf revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids [23].

Thin layer chromatography

Thin layer chromatography studies showed that the leaf extract of *A. tetracantha* leaves contain various secondary metabolites which were confirmed with the formation of bands (fig. 3). The solvent contains 100 ml petroleum ether, 11ml acetone and 1ml distilled water was used and one spot was identified and Rf value was 0.20. Similarly, TLC of ethanol extract of *M. indica* with solvent system Hexane: Ethyl acetate: Acetic Acid (4:4:2) 2 spots were visible and the Rf values were 0.23 and 0.75 respectively [24]. Similarly methanolic extracts of *Leucas aspera* in TLC showed 8 major bands with Rf values of 0.95, 0.81, 0.69, 0.58, 0.46, 0.38, 0.06 and 0.04 which corresponds to major compounds such as triterpenoids and steroids, phenolic compound and catechin, flavonoids-glycosides and mentione, saponin, terpene alcohols and quercetin, sterols and polylines [25].

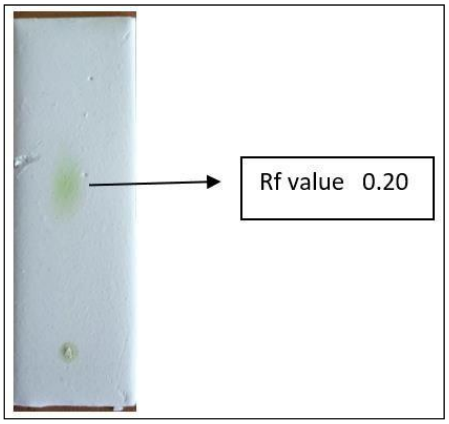

Fig 3: Thin Layer Chromatography using leaf extract of *A.tetracantha*

Table 1: Preliminary qualitative phytochemical analysis of leaf extracts of *A. tetracantha*

S.No	Phytochemicals	Hexane	Acetone	Methanol	Aqueous
1	Carbohydrates	-	-	-	-
2	Alkaloids	-	+	-	-
3	Steroids	+	-	+	-
4	Flavonoids	+	+	+	+
5	Terpenoids	-	-	-	+
6	Saponins	-	+	-	+
7	Tannins	+	-	+	+
8	Phenols	+	-	+	+
9	Leuco Anthocyanin	-	-	-	-
10	Coumarins	+	+	+	+
11	Glycosides	-	-	+	+

Conclusion

The results obtained in the present article indicated the pharmacological properties of the leaves extract of *A. tetracantha*. Screening of phytochemicals in given plant showed more positive result in aqueous leaf extract than the other extract. The phytochemical analysis and thin layer chromatography of *A. tetracantha* showed maximum amount of phytoconstituents. It also highlighted the medicinal value and used in treatments of some diseases.

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