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Analysis of different solvents systems used in phytochemical extraction of *Justicia adhatoda* L. –A potential medicinal plant

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Abstract

Solvent extraction is most frequently used technique for isolation of plant metabolites. 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. In the present study the effect of extraction solvents on Phytochemicals of various parts of *Justicia adhatoda* have been investigated using TLC technique. It is an attempt to correlate the effect of extraction solvents on Phytochemicals of various parts of *Justicia adhatoda* using TLC.

1. Introduction

Medicinal plants have been the subject of man's curiosity since time immemorial (Azwanida, 2015). They are being used by about 80 % of the world population for their primary health care (Cordell, 1993). In fact ancient man was totally dependent on medicinal plants for the treatment of various ailments (Cacace and Mazza, 2003).

Drugs from plants consist of entire plant or their parts like leaves, roots, fruits, seeds etc. Dried plants or plant parts and phytochemical been widely used for the preparation of phytomedicines in ayurvedic , allopathic , unani , siddha , homeopathic and folk medicines. The disease curing properties of plants are associated with their chemical constituents (Cosa *et al*., 2006). An integral part of Indian culture from Vedic ages (1500 – 800 BC) - ayurveda , mainly used plant based drugs for the treatments . Following the discovery of modern medicines in the 18th century , herbal medicines including ayurvedic ones suffered a setback. However , presently there has been an increasing interest for the plant based drugs because of the ready acceptance to local population , relative inexpensiveness and minimal side effects. The fascination of our holistic system of medicine especially ayurveda , which relies on the use of more than 7000 medicinal plants attained popularity not only in India but also abroad (Eloff, 1998). As a result of ever increasing demand for medicinal herbs , the supply of medicinal plants has dwindled . According to World Health Organization , these starting materials for medicinal preparations represent

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some 21000 plant species of which 70 – 90 % are obtained through commercial collection from wild habitat (2002). (Fabricant and Farnsworth, 2001). Plants form an important part of our everyday diet , and plant constituents and their nutritional value have been intensively studied for decades. In addition to essential primary metabolites, higher plants are able to synthesize a wide variety of low molecular weight compounds such as secondary metabolites. Plant secondary metabolites can be defined as compounds that have no recognized role in the maintenance of fundamental life process in the plants that synthesize them , but they do have an important role in the interaction of plant with its environment . The production of these compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stages of the plant (Lade *et al*., 2014).

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, they are highly potential in curing various ailments, which also provide health benefits for humans than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack etc. (Sasidharan *et al*., 2011). Extraction solvents have pronounced influence in the extraction of phytochemicals from medicinal plants. Considering all these an important medicinal plant *Justicia adhatoda* L. was selected for the present study to analyze the effect of different extraction solvents in the phytochemical extraction of selected medicinal plant (Spigno *et al*., 2007).

2. Materials and Methods

Sample collection and preparation

The plant sample of Justicia adhatoda L. was collected from Kozhikode district, Kerala and authenticated by available literature and Floras.

Plant material was cleaned well in running water, dried in hot air oven at 40°C and leaf, stem and root were separately ground to fine powder using electric blender. Powdered samples were kept away from sunlight in air tight containers at room temperature.

Chemicals used

Methanol and Hexane (Merck, India) were used as extraction solvents. Toluene, methanol and ethyl acetate (Merck, India) were used in TLC studies.

Extraction

Leaf, stem and root samples were extracted in Methanol and Hexane using reflux condenser. 3g of each sample was taken in a RB flask with 100mL of solvent and boiled for 3 hours. These extracts were filtered using Whatman No.1 filter paper and concentrated to 10mL on a water-bath. These extracts were kept in amber bottles and preserved in refrigerator.

Thin Layer Chromatography (TLC) studies

Thin Layer Chromatography (TLC) profiling of different extracts of leaf, stem and root of Justicia adhatoda was carried out on pre-coated silica gel plates (60 F_{254} Merck) using toluene, methanol and ethyl acetate as mobile phase in the ratio 7:3:1. The developed plates were observed under visible spectrum, UV 254nm and 366nm and observations were recorded. R_f values of each compounds visible as different bands were calculated using the following formula.

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plate}}$$

3. Results and Discussion

The chemical pattern of different extracts was compared in this investigation using TLC profiling. Variations were observed in terms of number of bands and band intensity which indicate the qualitative and quantitative divergence in chemical constituents (Fig. 1).

Stem

Methanolic and hexane extracts showed several unique bands. Bands of Rf = 0.44, 0.51, 0.52, 0.54 and 0.98 were seen only in methanolic extracts (under visible light). Whereas no bands were observed in hexane extracts (under visible light). Bands of Rf = 0.25, 0.50 and 0.95 were seen only in methanolic extracts (under UV 254nm). Whereas no bands were observed in hexane extracts (under UV254nm). Similarly specific bands for Methanolic and hexane extracts were observed under UV 366 nm also. The TLC data of stem extracts is given in Table-1.

Leaf

Methanolic and hexane extracts showed several unique bands. Bands of Rf = 0.07, 0.25, 0.41, 0.48, 0.51, 0.53, 0.57, 0.63, 0.65, 0.71, 0.77, 0.90 and 0.98 were seen only in methanolic extracts (under visible light). Whereas Bands of Rf = 0.93 was seen only in hexane extracts (under visible light). Bands of Rf = 0.22, was seen only in methanolic extracts (under UV 254nm). Whereas Bands of Rf = 0.63 and 0.92 were seen only in hexane extracts (under UV254nm). Similarly specific bands for Methanolic and hexane extracts were observed under UV 366 nm also. The TLC data of leaf extracts is given in Table-2.

Root

Hot and cold extraction methods showed several unique bands. Band of Rf = 0.27 was seen only in methanolic extracts (under visible light). Whereas no bands were observed in hexane extracts (under visible light). Band of Rf = 0.33 was seen only in methanolic extracts (under UV 254nm). Whereas no bands were observed in hexane extracts (under UV254nm). Similarly specific bands for Methanolic and hexane extracts were observed under UV 366 nm also. The TLC data of root extracts is given in Table-3.

In the presnt study toluene, methanol and ethyl acetate in the ratio 7:3:1 was the best solvent system for the separation. Disparities were observed in terms of number of bands and band intensity of the TLC profiles developed for different parts, such as leaf, stem

and root of *Justicia adhatoda* with various solvents like Hexane and Methanol which specify the qualitative and quantitative deviation in chemical constituents. Importance of solvent system and specific organs in the extraction of phytochemicals was reported earlier in various plants by Tomsone *et al* 2012; Li *et al* 2013 and Pliszka *et al* 2016.

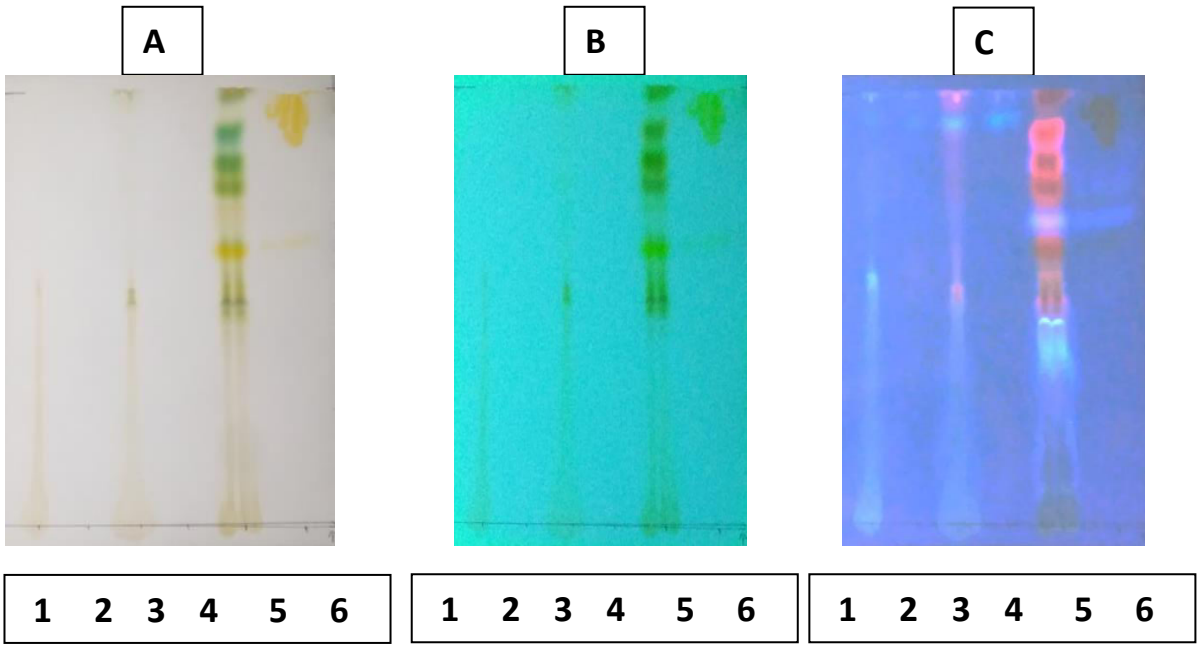

Fig. 1: Effect of extraction solvents on the phytoconstituents of different parts of *Justicia adhatoda* L. A. TLC Fingerprints under visible light, **B**. TLC Fingerprints under UV 254 nm and **C**. TLC Fingerprints under UV 366 nm. **1**. Root methanolic extract, **2**. Root hexane extract, **3**. Stem methanolic extract, **4**. Stem hexane extract, **5**. Leaf methanolic extract and **6**. Leaf hexane extract

Stem

Visible			254NM			366NM		
Rf	**Methanol**	**Hexane**	**Rf**	**Methanol**	**Hexane**	**Rf**	**Methanol**	**Hexane**
0.44	√	-	0.25	√	-	0.48	√	x
0.51	√	-	0.50	√	-	0.52	√	x
0.52	√	-	0.95	√	-	0.92	√	√
0.54	√	-	-	-	-	0.96	√	x
0.98	√	-	-	-	-	0.98	x	√

Leaf

Visible			254NM			366NM		
RF	**Methanol**	**Hexane**	**Rf**	**Methanol**	**Hexane**	**Rf**	**Methanol**	**Hexane**
0.07	√	X	0.22	√	x	0.44	√	x
0.25	√	X	0.63	X	√	0.48	√	x
0.41	√	X	0.92	X	√	0.53	√	x
0.48	√	X	-	-	-	0.56	√	x
0.51	√	X	-	-	-	0.60	√	x
0.53	√	X	-	-	-	0.64	√	x
0.57	√	X	-	-	-	0.71	√	x
0.63	√	X	-	-	-	0.76	√	x
0.65	√	X	-	-	-	0.81	√	x
0.71	√	X	-	-	-	0.84	√	x
0.77	√	X	-	-	-	0.87	√	x
0.90	√	X	-	-	-	0.92	√	x
0.93	x	√	-	-	-	0.93	x	√
0.98	√	X	-	-	-	0.95	√	x
-	-	-	-	-	-	0.98	√	x

Root

Visible			254NM			366NM		
RF	**Methanol**	**Hexane**	**Rf**	**Methanol**	**Hexane**	**Rf**	**Methanol**	**Hexane**
0.27	√	X	0.33	√	x	0.55	√	x
-	-	-	-	-	-	0.92	x	√
-	-	-	-	-	-	0.94	√	x
-	-	-	-	-	-	0.97	√	x
-	-	-	-	-	-	0.99	x	√

4. Conclusion

Medicinal plants, since time immemorial, have been used in almost all cultures as a source of medicine. Nature has been a source of medicinal agents for thousands of years and a large number of modern drugs have been derived from natural sources. Many of these drugs were isolated from plants based on the leads from their use in traditional or tribal systems of medicine. Traditional medicine, mostly based on plants derived products, has served as a source of alternative medicine, new pharmaceuticals and healthcare products. Medicinal plants are natural products and their chemical composition varies depending on several factors, such as botanical species, chemotype, the plant part used, time of harvest, geographic area and also processing, storage conditions, etc. This variability can result in significant differences in pharmacological activity. Conventional pharmaceutical products, herbal medicinal products may vary in composition and properties, and increasing reports of adverse reactions has drawn the attention of many regulatory agencies for the standardization of herbal formulations. Correct identification and quality assurance is an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy.

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. Polar solvents are frequently employed for the recovery of polyphenols from a plant matrix. 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. In the present study the effect of extraction solvents on Phytochemicals of various parts of *Justicia adhatoda* have been investigated using TLC technique. Toluene, methanol and ethyl acetate in the ratio 7:3:1 was the best solvent system for the separation. Disparities were observed in terms of number of bands and band intensity of the TLC profiles developed for different parts, such as leaf, stem and root of *Justicia adhatoda* with various solvents like Hexane and Methanol which specify the qualitative and quantitative deviation in chemical constituents. In conclusion, the present work was an attempt to correlate the effect of extraction solvents on Phytochemicals of various parts of *Justicia adhatoda* using TLC.

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