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Identification of genuine and adulterants (if any) in the collected samples of *Cymbopogon martini* (Roxb.)Wats.

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Abstract

Studies on adulteration practices will have to be taken up along with identification of the scares drugs. Conservation measures of their natural habitat, medicinal plant cultivation using various conventional propagation practices may have to be taken up in large scale. Taxonomic validation of plants at chemical level is a critical step for therapeutic preparations. TLC fingerprints of phyto-extracts can be used for the identification of plants. The chromatograms of extracts of different samples were compared in this investigation for TLC profiling. Variations were observed in terms of number of bands and band intensity which indicate the qualitative and quantitative divergence in chemical constituents. In order to ensure that original raw materials are used in preparation of herbal medicines commercial manufacturers have to cultivate the medicinal plants under their own management. This will ensure conservation of important medicinal plants as well as efficacy of the treatment.

1. Introduction

Drugs obtained from plants consist of entire plant or their parts leaves, roots, fruits, seeds, rind etc. Dried plants or plant parts and phytochemicals have been widely used for the preparation of phytomedicines in ayurvedic, allopathic, unani, siddha, homoeopathic and folk medicines. The disease curing properties of plants are associated with their chemical constituents. Ayurveda, an integral part of Indian culture from vedic ages (1500-800 B.C) mainly uses plant based drugs for the treatment of diseases. Following the discovery of modern medicines in the 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 populance, 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 (Sawada *et al*., 2016).

The National medicinal Plant Board (NMPB) , Department of AYUSH, Government of India during 2006-2007,revealed that the annual demand of botanical raw drug in the country has been estimated at 3,19,500metric tone for the year 2006 (Ved & Goraya, 2007). Global market of herbal medicine is hiking up every year. India is sitting on a gold mine of well

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recorded and well practiced knowledge of traditional medicine. One of the main problems is that the identification of plants is not yet too easy. Other aspect of it is that, even, if collected and identified correctly, their medicinal value and importance get changed with region and season. For example the herb collected in the spring might not be effective as may be in winter. Herbal medicines are to be effective as conventional medicines, but with less side effects (Street & Shillito, 1977).

India is one of the world's richest sources of medicinal plants because of its rich geographical' diversity, varied climatic and ecological features. About 60% of Indian population depends mainly on ayurvedic medicines for the treatment of common diseases (Nair *et. al*., 1992). Presently, the traditional herbal medicines, both nationally and internationally are receiving considerable attention from pharmaceutical industries. The scientific study of traditional medicines, derivatives of drugs, through bioprospection and systematic conservation, domestication and cultivation of the medicinal plants thus assumes great importance in today's context when more people need safe and effective medicine at affordable rate.

It has been estimated that about 30% of pharmaceuticals are derived from green plants and this percentage has risen considerably in recent years. The market for whole plant preparations, often sold as complimentary or safer alternative medicines has also been increasing (Saxena, 2002). In most industrialized countries the use of medicinal plants has increased dramatically in the last decade (Rajendra & D'souza, 1999). Medicinal plants therefore form an important part of international commerce. Present global market of medicinal plants or their products is that of Rs.360000 crore annually. Of these Indian share is pegged at Rs.2800 crore and is growing by 7% annually. Trade in medicinal plants between developed and developing countries is expected to touch $500 billion by the turn of the century (Abraham, 2002). As a result of ever-increasing demand for medicinal herbs, the supply of the medicinal plants has dwindled. According to World Health Organization, these starting materials for medicinal preparations represent some 21000 plant species of which 70 to 90 percent are obtained through commercial collection from wild habitat (WHO, 1978). Even now, most of the plants used in medicines are collected from their wild habitats and only some species used in larger quantities are cultivated systematically.

Many medicinal plants, which were ignored in the past years, have been over exploited in recent years. The plant collectors increase in number, but the number of plants still found in the wild is progressively declining (Rajendra & D'Souza, 1999). Collected plant drugs, especially those wild crafted and traded under the vernacular name, are very prone to mislabeling, making an analytical determination of identity important. Plant Collections from the wild were usually subjected to either intentional or unintentional adulterations (Santhosh *et al*. 2015). Substitution of plants could occur due to more than

one species sharing the same vernacular name and therefore leading to confusion for the collectors. It could also occur due to the inability of the collector to differentiate two or more species because of their close external resemblance (Srirama *et al*. 2010; Sreelatha and Kameshwari, 2017). Species changeover may harmfully affect user healthiness as it could cause severe allergies and will not have the intended effect (Santhosh *et al*. 2015, 2016; Srirama *et al*. 2017). Visual recognition of species adulteration in the raw herbal trade is often complicated, as the plants are usually in a dry condition and do not retain the original features of the plant (Uniyal and Joshi, 1993). Thus it has become imperative to develop methods to identify adulteration and substitution of important medicinal plants used in various systems of medicine.

Objectives of the present study are

- Collection of *Cymbopogon martini* (Rxb.), Watson / pamarosa / Dyamakah from various market places.
- Identification of genuine and adulterants (if any) in the collected samples of *Cymbopogon martini* (Rxb.), Watson / pamarosa / Dyamakah samples using Thin Layer Chromatographic (TLC) analysis of hexane extract/ methanol extract.

2. Materials and Methods

Sample collection

Samples of of *Cymbopogon martinii* (Rxb.), Watson / pamarosa / Dyamakah were collected from various market sources. Collected plant materials were cleaned and dried in hot air oven at 40°C and ground to fine powder using electric blender. Powdered samples were kept in air tight containers away from sunlight at room temperature.

Chemicals used

Methanol and hexane (Merck^R) were used as extraction solvents. Toluene, methanol and Ethyl acetate (Merck^R) were used in TLC studies.

Extraction

Powdered rhizome of different samples was extracted in Methanol and Hexane using cold extraction method. 3g of each sample was taken in a conical flask with 100mL of solvent (Methanol / hexane) and kept for 24 hours with frequent agitation. The extracts were filtered using Whatman No.1 filter paper and concentrated to 10mL using a water-bath. These extracts were kept in amber bottles in refrigerator.

Thin Layer Chromatography studies

Thin Layer Chromatography (TLC) profiling of methanolic extracts of *Cymbopogon martinii* was carried out on pre-coated silica gel plates (60 F_{254} Merck) using toluene, ethyl acetate and methanol as mobile phase in the ratio 7:3:1. Thin Layer Chromatography (TLC) profiling of hexane extracts of *Cymbopogon martinii* was carried out on pre-coated silica gel plates (60 F_{254} Merck) using toluene and ethyl acetate as mobile phase in the ratio 9:1.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.

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

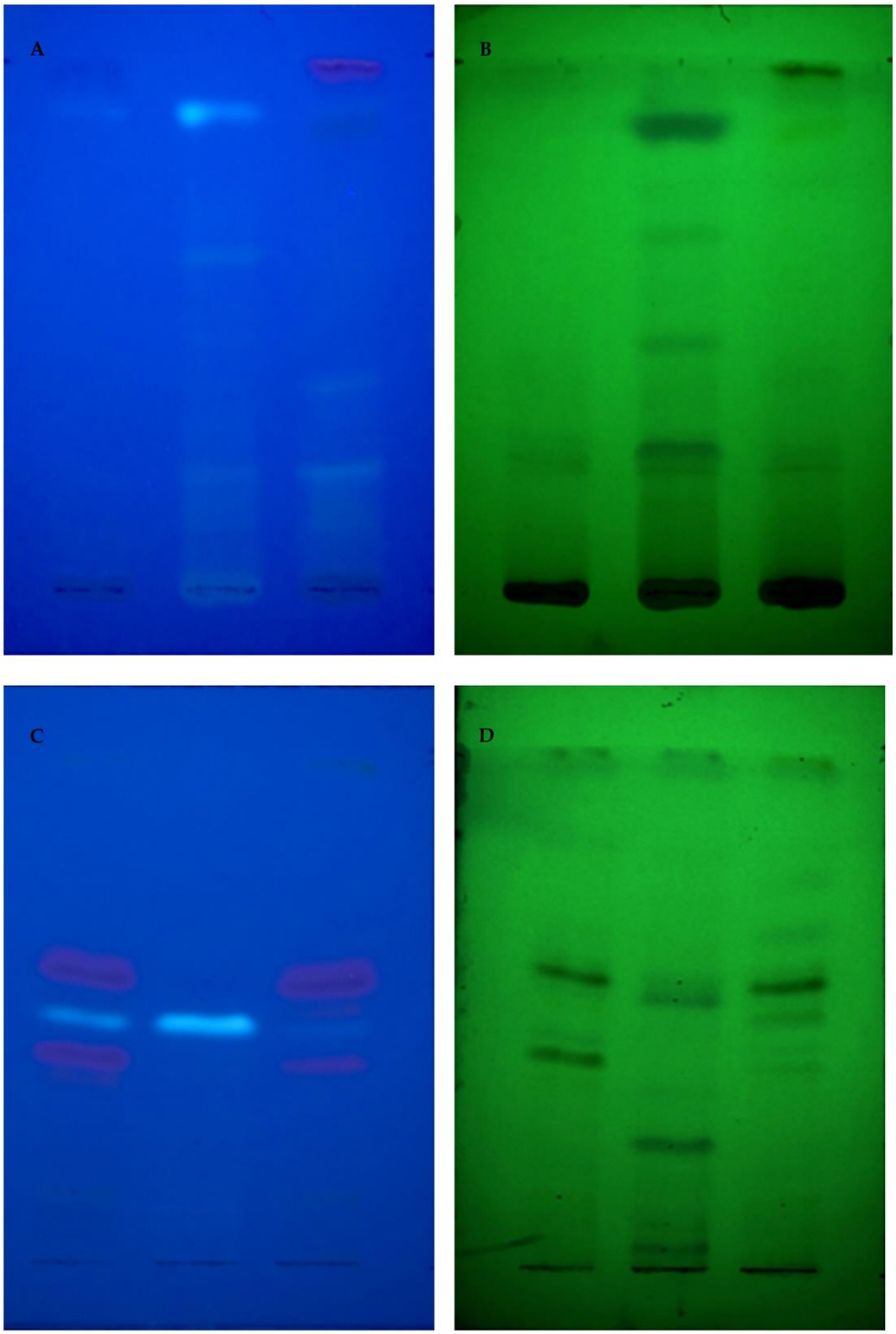

Figure 1: A. Methanolic extract of *Cymbopogon* samples under UV 366nm, B. Methanolic extract of *Cymbopogon* under UV 254nm, C. Hexane extract of *Cymbopogon* samples under UV 366nm and D. Hexane extract of *Cymbopogon* samples under UV 254nm. Track 1 and 2 are *Cymbopogon* samples collected from market and C is the authentic sample of *Cymbopogon martini*.

3. Result and Discussion

Taxonomic validation of plants at chemical level is a critical step for therapeutic preparations. TLC fingerprints of phyto-extracts can be used for the identification of plants. The chromatograms of extracts of different samples were compared in this investigation for TLC profiling. Variations were observed in terms of number of bands and band intensity which indicate the qualitative and quantitative divergence in chemical constituents.

Hexane extract

UV 366nm

Compounds at Rf. 0.48, 0.84, 0.86 were present in all the collected samples and genuine plant. Compounds with Rf. 0.3, 0.21, 0.45 and 0.7 were found only in sample 2.Compounds with Rf. 0.12, 0. 37 and 0. 51 were present only in sample 1 and genuine sample of *Cymbopogon martinii* (Table 1, Figure 1C)

UV 254nm

Compounds at Rf. 0.34 0.48 0.51 were present in sample 1 and genuine sample of *Cymbopogon martinii*. Compound with Rf.0.39 was found only in sample 2 and genuine plant. Compound with Rf. 0.42 was present only in genuine sample of *Cymbopogon martinii*(Table 2,Figure 1D)

Methanolic extract

UV 366nm

Compound with Rf. 0.17 was present in all samples.

Compound with Rf.0.8 was presnt in sample 1 and sample 2.

Compound with 0.86 was presnt in sample 1 and genuine sample of *Cymbopogon martinii*.

Compound with Rf. 0.3 was present in sample 1 and genuine sample of *Cymbopogon martinii*.

Compound with Rf.0.54 was present only in genuine sample of *Cymbopogon martinii*(Table 3,Figure 1A)

UV 254nm

Compound with Rf. 0.2 0 and 0.24 were present in all samples.

Compound with Rf.0.8 was presnt in sample 1 and sample 2.

Compound with 0.86 was presnt in sample 1 and genuine sample of *Cymbopogon martinii*.

Compounds with Rf. 0.4 0.58 0.7 5 and 0.78 were present only in sample 2 (Table 4,Figure 1B).

Due to the phytochemical similarity with taxonomically identified ***Cymbopogon martinii***, sample 1 is identified as the genuine. Similar line of work for identifying genuine market samples of drug using Phytochemical studies have been reported by several authors (Purohit *et al*., 2010; Thani *et al*., 2018).

Table 1. Comparitive TLC analysis of hexane extract *Cymbopogan martinii* and its market samples at 366nm.

Sl.No	rf	Sample 1	Sample 2	*Cymbopogan martinii*
1	0.3	0	1	0
2	0.7	0	1	0
3	0.12	1	0	1

4	0.21	0	1	0
5	0.31	1	0	0
6	0.34	1	0	1
7	0.37	1	0	1
8	0.45	0	1	0
9	0.48	1	1	1
10	0.51	1	0	1
11	0.56	0	0	1
12	0.84	1	1	1
13	0.86	1	1	1

Table 2. Comparitive TLC analysis of hexane extract of *Cymbopogan martinii* and its market samples at 254nm .

Sl.No	Rf value	Sample 1	Sample 2	*Cymbopogan martinii*
1	0.34	1	0	1
2	0.39	0	1	1
3	0.42	0	0	1
4	0.48	1	0	1
5	0.51	1	0	1

Table 3. Comparitive TLC analysis of methanol extract of *Cymbopogan martinii* and its market samples at 254nm.

Sl.No	Rf	Sample 1	Sample 2	*Cymbopogan martinii*
1	0.2	1	1	1
2	0.24	1	1	1
3	0.34	0	0	1
4	0.4	0	1	0
5	0.58	0	1	0
6	0.75	0	1	0
7	0.78	0	1	0
8	0.86	1	0	1

Table 4. Comparitive TLC analysis of methanol extract of *Cymbopogan martinii* and its market samples at 366nm

Sl.No	Rf	sample 1	sample 2	*Cymbopogan martinii*
1	0.17	1	1	1
2	0.3	0	0	1
3	0.54	0	1	0
4	0.76	0	0	1
5	0.8	1	1	0
6	0.86	1	0	1

4. Conclusion

Accurate identification of botanical source of herbal medicines is a major problem till date in the field of traditional medicine. Substitution of the herbs is the need of the hour with more than300 medicinal plants becoming red listed. Adulteration and Substitution are different. The most essential criteria for substitution is the pharmacological activity rather than morphology or phytoconstituents. Adulteration is a malpractice not only done intentionally but accidentally due to involvement of untrained personnel in collection and trade .Controversy about authentic botanical source of medicinal plants dealt in classical Ayurvedic texts and problem regard-ing substitution and adulteration should be resolved by integrated research and those sources should be validated which have more potency for described pharmaco-logical activities. Studies on adulteration practices will have to be taken up along with identification of the scares drugs. Conservation measures of their natural habitat, medicinal plant cultivation using various conventional propagation practices may have to be taken up in large scale. In order to ensure that original raw materials are used in preparation of herbal medicines commercial manufacturers have to cultivate the medicinal plants under their own management. This will ensure conservation of important medicinal plants as well as efficacy of the treatment.

5. References

Abraham, V. (2002). Showcasing herbal remedies. In: Mathew M (Ed.) The Week.20(48), Manorama Publication, Kerala, India, pp: 39

Nair, K.V., Nair, A.R. and Nair, C. P. R. (1992). Techo- economic data, cultivation and preservation of some South Indian medicinal plants, *Aryavaid.*, **5:** 238-240.

Purohit, H., Nautiyal, B.P. and Nautiyal, M.C. (2010). . Interpopulation variation in Picrorhiza kurrooa Royle ex Benth-step towards identifying genetic variability and elite strains for crop improvement study. *Amer. J. Plant Physiol.*, **5(4):** 193-203.

Rajendra, K. and D'souza, L. (1999). *In vitro* propagation of ayurvedic plants . In : Khan I.A. and Khanum A. (Eds.) *Role of Biotechnology in Medicinal and Aromatic Plants* .Vol.II Ukaaz Publication, Hyderabad, India, pp: 207-237.

Santhosh, K., Krishna, V., Seethapathy, G.S., Senthilkumar, U, Ragupathy, S., Ganeshaiah, K.N., Ravikanth, G. and Uma Shaanker, R. (2015). DNA barcoding to assess species adulteration in raw drug trade of "Bala" (genus: Sida L.) herbal products in South India. *Biochem Sys Ecol.,* **61:**501–509.

Sawada, S., Okano, J., Imamoto, R., Yasunaka, Y., Abe, R., Koda, M., Murawaki, Y. and Isomoto, H. (2016). Preventive Effect of Geraniol on Diethylnitrosamine-Induced Hepatocarcinogenesis in Rats. *Yonago acta medica*, **59(1):** 37–43.

Saxena, P. K. (2002). Adulterants and substitutes in Ayurvedic formulations. *Pl . Cell Tiss. Org. Cult*., 68: 213.

Sreelatha, K.C. and Kameshwari, S. (2017). Morphological and cytogenetic studies in wild species of Cymbopogon martinii (Roxb.) Wats from Kodaicanal (T.N.). *J. Med. Plants Stud*., **5(2):** 175-179.

Srirama, R., Senthilkumar, U., Sreejayan, N., Ravikanth, G., Gurumurthy, B.R., Shivanna, M.B. and Uma Shaanker, R. (2010). Assessing species admixtures in raw drug trade of Phyllanthus, a hepato-protective plant using molecular tools. *J. Ethnopharmacol.,***130:** 208–215

Street, H. E. and Shill to, R.D. (1977). Nutrient media for plant organ, Tissue and cell culture.Rechcigl M (ed) CRC. *Hand book in nutrition and food*, 4 CRC Press Boca Raton.

Thani, P.R., Yash ,Pal Sharma and Prashid, K. (2018). Phytochemical studies on Indian market samples of drug "Kutki" (*Picrorhiza kurroa* Royle ex Benth). *Res. J. Agricult. Forest. Sci.*, **6(2):** 1-5.

Uniyal, M.R. and Joshi, G.C. (1993). Historical view of the basic principles of the identification of controversial drugs, problems and suggestions. *Sachitra Ayurved.*, **45(7):** 531-536.

Ved, D.K. and Goraya, G. (2007). . Demand and supply of medicinal plant in India. NMPB, New Delhi & FRLHT, Bangalore, India.

WHO. (1978). WHO resolutions WHA . 31.33.1978 : WHA 40.33.