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Research Article

## Standardization of free radical scavenging activity of some indigenous medicinal plants by using thin layer chromatography

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**Abstract:** Thin layer chromatography is basically used to separate out plants active constituents, various dyes and chemical analysis. The present study is designed to find out free radical scavenging property with the help of thin layer chromatography. For conducting this experiment 15 medicinal plants of local region were selected. Collected plants were shade dried, grind to a coarse powder and subjected to extraction with Soxhlet apparatus followed by concentration under reduced pressure by rotatory evaporator. The Methanolic plant extract was dissolved in methanol and 1 $\mu$ l of extract of 50mg/ml concentration was spotted on the aluminum silica plate (1.05554.0007 spectrum, Merck Germany). It was then subjected to run in Toluene: ethyl acetate (70:20) solvent mobile phase. Run plate was removed, dried and calculated R<sub>f</sub> values for every separated active constituent. The developed plate sprayed with DPPH (free radical scavenger) reagent at 0.1mM concentration and kept for drying few minutes. After drying the zone of inhibition seen in developing plate. Formation of zone of inhibition is due to the reaction between hydroxyl ions of DPPH and test plant extracts. Results revealed that 5 plants like *Azadirachta indica* leaves, *Dalbergia sissoo* leaves, *Citrus medica* leaves, *Ocimum sanctum* leaves and *Prunus persica* leaves were showed good free radical scavenging activity out of 15 medicinal plants. From the above, it can be concluded that plants having good free radical scavenging property can be used as formation of medicine to preventing ageing and for other stressful condition.

**Keywords:** Free radical scavenging property, TLC, Indigenous plants, R<sub>f</sub> value.

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### 1. Introduction

There is extensive evidence to implicate free radicals in the development of degenerative diseases (Cross, 1987). Free radicals have been implicated in causation of ailments such as diabetes, liver cirrhosis and nephrotoxicity (Marx, 1987). Together with other derivatives of oxygen, they are inevitable byproducts of toxic oxygen derivatives is considered to be a universal feature of stress condition. Oxidative stress has been linked to cancer, ageing, Atherosclerosis, ischemic injury, inflammation and neurogenerative disease. Plants and other organisms have evolved a wide range of mechanism to contend with this problem, with a variety of antioxidant molecules and enzymes. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species. Hence, present study is designed to standardize antioxidant potential of some indigenous plants with the help of thin layer chromatography.

### 2. Material & methods

#### 2.1 Collection & preparation of plant extracts

For conducting present study we collected 15 indigenous plants *Prunus persica* leaves (Peech), *Syzygium cumini* leaves (Jamun), *Litsaea anthapoly* bark (Pojo), *Cassia tora* leaves (Takala), *Annona squamosa* leaves (Sharifa), *Dalbergia sissoo* leaves (Sheesham), *Argemone mexicana* leaves (Kateli), *Eucalyptus* leaves (Nilgiri), *Ocimum sanctum* leaves (Tulsi), *Ficus benghalensis* leaves (Banyan), *Hibiscus rosa sinensis* leaves (Gurhal), *Vitex trifolia* leaves (Safed sambhalu), *Citrus aurantium* leaves (Lemon), *Adhatoda vasica* leaves (Vasaka) and *Azadirachta indica* leaves (Neem) from local area (Mathura-Agra) in different-different suitable seasons. The collected plant materials were authenticated with related taxonomy in literature. All the collected plant materials were dried in shade and grind to a coarse powder. The coarse powder (100gm) of the shade dried

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(different) plant parts was exhaustively extracted using methanol in Soxhlet extractor for a period of 22 hours, as per standard methods. The Prepared liquid extract was concentrated by vacuum rotary evaporator (Heidolph, Japan), in which the temperature of water bath and Rota cool was 35°C and 4°C with 147 bar vacuum pressure.

## 2.2 TLC profile

All the methanolic plants extract dissolved in methanol at the same concentration (50mg/ml) and applied 1µl on thin layer plate (Aluminum silica plate) and subjected to run for alkaloids in Toluene: Ethyl acetate (70:20) solvent system. Run plate was removed, dried and observed in the ultraviolet chamber. Rf values for each separated ingredients were noted. This run plate was spread with DPPH solution of standard concentration (0.1mM). After drying DPPH solution, the zone of inhibition had seen on developed plate. Formation of zone of inhibition was due to the reaction between hydroxyl ions of DPPH and test plant extracts (Shrififar *et al.*, 2003).

In the general color development of the run, thin layer chromatography plate was used for separated chemical constituent (alkaloids). Out of 15 plants, 5 were positive for colour development at visible and ultraviolet light. In *Prunus persica* plant leaves 2 spots for grey colour, 1 for green, 2 for yellow, 1 for light green, 1 for greenish yellow, 1 for yellowish brown and other 1 for yellowish green colour at visible light, while all spots are red at ultraviolet light. In *Ocimum sanctum* plant leaves 2 spots for green colour, 2 for grey and other one for yellowish colour at visible light, while all spots are red at ultraviolet light. In *Azadirachta indica* plant leaves 2 spots for green colour, 2 for grey, 2 for yellowish and other for brownish green colour at visible light, while all spots are red at ultraviolet light. In *Citrus medica* plant leaves 4 spots for green colour, 2 for yellow and another for grey colour at visible light, while 5 spots are red and 2 spots are sky blue at ultraviolet light. In *Dalbergia sissoo* plant leaves 3 spots for green colour, 2 for yellow, 1 for grey, 1 for sky blue and other for yellowish green colour at visible light, while all are red at ultraviolet light.

Table 1. Rf values of most potent plants.

Plant extract	No. of spots	Rf values	Colour in visible light	Colour in ultralight
<i>Prunus persica</i> (Leaves)	9	Rf1=0.15	Yellow	Red
		Rf2=0.34	Green	Red
		Rf3=0.36	Greenish yellow	Red
		Rf4=0.44	Grey	Red
		Rf5=0.60	Yellowish brown	Red
		Rf6=0.68	Grey	Red
		Rf7=0.69	Yellowish green	Red
		Rf8=0.74	Yellow	Red
		Rf9=0.89	Light green	Red
<i>Ocimum sanctum</i> (Leaves)	5	Rf1=0.23	Green	Red
		Rf2=0.25	Green	Red
		Rf3=0.49	Yellowish green	Red
		Rf4=0.54	Grey	Red
		Rf5=0.62	Grey	Red
<i>Azadirachta indica</i> (Leaves)	7	Rf1=0.36	Yellowish green	Red
		Rf2=0.42	Green	Red
		Rf3=0.46	Grey	Red
		Rf4=0.54	Brownish green	Red
		Rf5=0.59	Yellowish green	Red
		Rf6=0.67	Green	Red
		Rf7=0.72	Grey	Red
<i>Citrus medica</i> (Leaves)	7	Rf1=0.22	Green	Red
		Rf2=0.34	Grey	Red
		Rf3=0.44	Yellow	Red
		Rf4=0.50	Green	Sky blue
		Rf5=0.56	Green	Red
		Rf6=0.62	Green	Sky blue
		Rf7=0.82	Yellow	Red
<i>Dalbergia sissoo</i> (Leaves)	8	Rf1=0.20	Yellow	Red
		Rf2=0.23	Green	Red
		Rf3=0.33	Green	Red
		Rf4=0.36	Sky blue	Red
		Rf5=0.46	Yellowish green	Red
		Rf6=0.58	Green	Red
		Rf7=0.66	Grey	Red
		Rf8=0.80	yellow	Red

The results revealed that plants having good free radical scavenging property could be used as formation of medicine in preventing ageing and for other stressful condition.

### 3. Summary

Free radical scavenging activity of some indigenous medicinal plants was assessed by thin layer chromatography. The collected plant material was shade dried and goes to the extraction by Soxhlet apparatus followed by concentration under reduced pressure by rotary evaporator. Prepared methanolic plant extract was tested at 50mg/ml concentration, which revealed Rf values varied from 0.15 to 0.89 in *Prunus persica* plant leaves, 0.23 to 0.62 in *Ocimum sanctum* plant leaves, 0.36 to 0.72 in *Azadirachta indica* plant leaves, 0.22 to 0.82 in *Citrus medica* plant leaves and 0.20 to 0.80 in *Dalbergia sissoo* plant

leaves, while the rest of the plants showed no or poor activity.

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