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## Phytochemical Screening and High Performance TLC Analysis of Some Cucurbits

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**Abstract:** Fruit extracts of *Lagenaria siceraria* (Molina) Standl., *Luffa cylindrica* (Linn) Roem. and *Cucurbita maxima* (Duchesne) Lam. was carried out for the phytochemical screening and High Performance TLC analysis to ascertain relative relationship between active compounds. Present study revealed the presence of alkaloids, flavonoids, saponins and steroids in moderate concentration. High Performance TLC on Silica gel 60 F<sub>254</sub> TLC aluminium sheet was used to separate the active compound present in the extracts. TLC sheet was scanned at 200 nm and revealed that the retention factor (Rf), 0.05 and 0.26 of active compounds were common in all three vegetables. Another two active compounds of Rf 0.40 and 0.63 were common in *Lagenaria siceraria* and *Luffa cylindrica*. However, Rf 0.20 was common in *Lagenaria siceraria* and *Cucumis maxima*. *Lagenaria siceraria* showed highest peaks, having 44.4% paired affinity with *Luffa cylindrica* and 28.6% with *Cucumis maxima*, respectively.

**Key words:** Phytochemical, high performance TLC, retention factor, pair affinity

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### INTRODUCTION

Cucurbits are dietary vegetable belongs to family Cucurbitaceae, which is primarily comprised species consumed as food worldwide. Cucurbits are an excellent fruit in nature having composition of all the essential constituents required for good health of humans (Rahman, 2003; Duke, 1999; Chopra *et al.*, 1992; Pawar *et al.*, 1985). Phytochemicals screening of dietary vegetables and fruits are essential, because it have been used for the maintenance of health and prevention of chronic diseases (Aggarwal and Shishodia, 2006). It contains large range of secondary metabolites, belongs to different chemical families and possesses distinct biological activities (Rizvi *et al.*, 2009). The attraction of pharmaceutical companies and researchers for elucidation of bioefficacies and provide knowledge for the advancement of phytomedicine. For example; some cucurbits like *Lagenaria siceraria* (Molina) Standl. and *Luffa cylindrica* (Linn) Roem have a Ribosome inactivating protein (RIPs), which is well known for the array of biological activities and possess anti-viral, anti-tumor and immunomodulatory activities (Islam *et al.*, 1990; Ng *et al.*, 1992; Gao *et al.*, 1994; Wang and Ng, 2000). Sapogenins isolated from the *Luffa cylindrica*, showed immunomodulatory activity (Khajuria *et al.*, 2007). Cucurbitacins predominantly found in the cucurbitaceae family, having great interest because of the wide range of biological activities like antioxidant, anti-inflammatory and inhibit the proliferation of cancer cells (Tannin-Spitz *et al.*, 2007). Moreover, due to the prevalence of chronic degenerative diseases worldwide, the availability

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information of phytomedicine will help individuals to make choices in the consumption of foods that could protect them from such chronic diseases. Present study made a phytochemical screening and High Performance TLC fingerprinting for the comparative analysis of compounds in commonly used three cucurbits vegetables.

## MATERIALS AND METHODS

### Materials

The cucurbits vegetables, *Lagenaria siceraria* (Molina) Standley (Bottle gourd), *Luffa cylindrica* (Linn.) Roem (Sponge gourd) and *Cucurbita maxima* (Duchesne) Lam (Pumpkin) were purchased from local market in month of February. It was identified by the Departmental Botanist and vouchers of specimen were stored in GBL, Department of Biosciences, Jamia Millia Islamia, New Delhi. This study was conducted from 2008 to 2009.

### Chemicals

Silica gel 60 F<sub>254</sub> TLC aluminium sheet (20×20 cm), HPLC water, Methanol, n-Butanol, Acetic acid, Formic acid, Ammonia hydroxide, Lead acetate, Glacial acetic acids, Mercuric chloride, Potassium iodide, Sublime iodide, NaOH, Sulphuric acids were purchase from Merck.

### Extraction

The fruits were cleaned with distilled water and juice was extracted by Juicer (Morphy Richards). The extracts were filtered through muslin cloth and centrifuged at 5000 rpm for 15 min. It was lyophilized into powder and stored in vacuum desiccators for further analysis.

### Phytochemical Analysis

The lyophilized powder of fruit extracts of cucurbits vegetables were screened for phytochemical constituents using standard procedures (Harborne, 1993; Sofowora, 1993; Trease and Evans, 2002; Kumar *et al.*, 2009; Olawale-Abulude, 2007). The experiments were performed in triplicates and solvents were used as control.

### High Performance TLC Study

Chromatography was performed on silica gel 60 F<sub>254</sub> TLC aluminium sheet (20×20 cm). Concentration of samples (10 mg mL<sup>-1</sup>) were applied as 6 mm wide bands using Camag Linomat 5 automated TLC applicator with nitrogen flow providing delivery speed of 150  $\mu$ L sec<sup>-1</sup> through the syringe. After completion of sample application, the plate was developed in a Camag Twin through glass tank presaturated with mobile phase of n-Butanol: Acetic acid: Water (4:1:5) for one hour. The TLC runs was performed under laboratory conditions. After development, the plates was taken off and dried. The spots were quantified using a Camag TLC Scanner model 3 equipped with Camag Wincats software. The scanning procedure was programmed as, speed 20 mm sec<sup>-1</sup>, slit width 6.0×0.45 mm and wave length ( $\lambda_{max}$ ) 200 nm. The retention factors (Rf) and % area was automated calculated by the Wincats software. R<sub>f</sub> is defined as the distance travelled by the compound divided by the distance travelled by the solvent.

### Analysis of High Performance TLC Finger Printing

The High Performance TLC peaks were analyzed by the method adopted by Ellison *et al.* (1962). It was followed to make the suitable comparisons in the form of

qualitative relationships of active compounds on the basis of their biochemical affinities. Values of Paired Affinity (PA), Group Affinity (GA) and Isolation Value (IV) were calculated as equation 1, 2 and 3, respectively. The HPTLC fingerprints also have been successfully used to distinctively identify the different phytochemical constituents on the basis of their Retention Factors (Rf) (Sunita and Abhishek, 2008; Ferreira *et al.*, 2009).

$$PA = \frac{\text{Spots common in species A and B}}{\text{Total spots in A and B}} \times 100 \quad (1)$$

$$GA = \text{Total PA value} + 100 \quad (2)$$

$$IV = \frac{\text{No. of unique spots in a species}}{\text{Total No. of spots in all species}} \times 100 \quad (3)$$

## RESULTS AND DISCUSSION

The result of the phytochemical screening is presented in Table 1, different standard test were performed and it was revealed the presence of alkaloids, flavonoids, saponins and steroids in moderate concentration in all three vegetables. However, trace amount of tannins, glycosides and anthraquinone were noticed. It was concluded that the cucurbits vegetable have not only essential nutrients, they also contains secondary metabolites of distinct groups. Among the effective ingredients, flavonoids are one of the most popular compounds; possess a variety of biological activities at nontoxic concentrations. The roles of dietary flavonoids in cancer prevention have been widely discussed (Ren *et al.*, 2003; Aggarwal and Shishodia, 2006). It have been reported effective in reducing blood lipid, as an anti-oxidative, in assimilating cholesterol, inhibiting thrombosis, dilating the coronary artery, etc. (Hollman and Katan, 1999; Di Carlo *et al.*, 1999). High Performance TLC on Silica gel 60 F<sub>254</sub> TLC aluminium sheet was used to separate the active compound present in the extracts. The TLC sheets was scanned at  $\lambda_{\text{max}}$  200 nm and revealed 10 peaks of *Lagenaria siceraria*, 8 peaks of *Luffa cylindrica* and 6 peaks of *Cucumis maxima*, respectively. The peaks were used for comparative analysis and revealed the active compounds of Rf 0.05 and 0.26 found in all three sps. However, active compounds of Rf 0.40 and 0.63 were common in *Lagenaria siceraria* and *Luffa cylindrica*. Similarly, Rf 0.20 was common in *Lagenaria siceraria* and *Cucumis maxima*. It was concluded that some phytochemicals in cucurbits sps were commonly present in all genus and some were found species specific. The concentration of active compounds was assessed by the % peak area (Fig. 1). The

Table 1: Phytochemical analysis of cucurbits by different standard methods

Phytochemical	Name of test	Colour observed	<i>Lagenaria siceraria</i>	<i>Luffa cylindrica</i>	<i>Cucumis maxima</i>
Alkaloid	Wagner	Pale yellow	++	+	+
	Mayer	Brown ppt.	++	++	+
Flavonoids	NaOH	Deep yellow	++	++	+
	NaOH/H <sub>2</sub> SO <sub>4</sub>	Colourless	++	++	++
	AlCl <sub>3</sub>	Yellow	+	+	++
Steroids	Salkowaski	Red colour	+	+	+
Glycoside	H <sub>2</sub> SO <sub>4</sub>	Dark brown	+	+	+
	Keller Killiani	Brown ring	-	-	-
Tannin	Braemer	Dark blue	-	-	-
	Iodine	Faint bluish	+	+	-
Anthraquinones	Bornträger	Deep red	+	+	-
Saponin		Frothing	++	++	++

-: Negative (absent), +: Positive (slightly present), ++: Positive (moderately present)

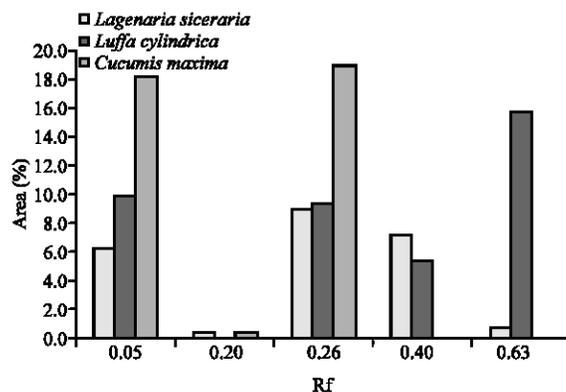


Fig. 1: Common active compounds its concentration

Table 2: Paired affinity, group affinity and isolation values of three cucurbits

Cucurbits	Paired affinity (PA)			Group Affinity (GA)	Isolation Value (IV)
	<i>Luffa cylindrica</i>	<i>Cucumis maxima</i>	<i>Lagenaria siceraria</i>		
<i>Lagenaria siceraria</i>	44.4	37.5	-----	181.94	20.83
<i>Luffa cylindrica</i>	-----	28.6	44.4	173.02	16.67
<i>Cucumis maxima</i>	28.6	-----	37.5	166.07	12.50

concentration of active compounds of Rf 0.05 and 0.26 were in order of *Cucumis maxima*>*Luffa cylindrica*>*Lagenaria siceraria*. Active compounds of Rf 0.40 was found in highest concentration in *Lagenaria siceraria* than the *Luffa cylindrica*. Hence, at Rf 0.63, *Luffa cylindrica* contained highest concentration than the *Lagenaria siceraria*. The peaks were also used for the evaluation of Paired Affinity (PA), Group Affinity (GA) and Isolation Value (IV) (Table 2). Highest PA value 44.4% was found between *Lagenaria siceraria* and *Luffa cylindrica* and lowest PA value 28.6% was found between *Luffa cylindrica* and *Cucumis maxima*. The PA value between *Lagenaria siceraria* and *Cucumis maxima* was 37.5%. These values predict the close relationship of *Lagenaria siceraria* with *Luffa cylindrica* than the *Cucumis maxima*. The relationships were also confirmed through the group affinity and isolation values of these cucurbits vegetables (Table 2). These finding supported that some of the active compound like saponins, cucurbitacin glucosides and cucurbitacines are commonly present in the cucurbits species (Khajuria *et al.*, 2007; Tannin-Spitz, 2007; Chen *et al.*, 2005). Therefore, further needed to isolate the separated compound to elucidate the chemical structure and its bioefficacies.

## CONCLUSIONS

The commonly used cucurbits vegetables, Bottle gourd, Sponge gourd and Pumpkin have not only essential nutrients, they also contain secondary metabolites such as alkaloids, flavonoids, glycosides, steroids and saponins. The essences of these metabolites are beneficial for maintenance of human health and chronic degenerative diseases. HPTLC fingerprinting revealed the presence of some common compound presence in the all three cucurbits vegetables and some are restricted to the species. There is further need of structural elucidation and bioefficacies of these active compounds.

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