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DEVELOPMENT OF QUALITY STANDARDS AND PHYTOCHEMICAL ANALYSIS OF *PEUCEDANUM GRANDE* C.B. CLARKE.

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ABSTRACT

Peucedanum grande is a succulent herb; about a meter in height. The fruits are used in medicine and have been reported to possess important biological properties. The objective of the present investigation was the development of quality standards and phytochemical analysis of *P. grande*. This included morphological and histological characters, pH of aqueous solution, ash values, extractive values, successive extractive values, and loss on drying, fluorescence analysis, preliminary phytochemical screening and HPTLC finger printing profile of secondary metabolites. The findings of this study might be useful to supplement information in regard to its identification parameters and laying down pharmacopoeial standards; as standardization of herbal medicines is essential and is the need of the today.

Key words: *Peucedanum grande*, extracts, phytochemical screening, HPTLC.

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INTRODUCTION:

Peucedanum grande C.B. Clark is a succulent herb, about a meter or more in height occurring gregariously on the Western Ghats and the hills of Deccan plateau, belongs to Apiaceae, Umbelliferae family. Ten species occur in India. *Peucedanum grande* have several name like Duku, Baphalle, Wild carrot, Hingupatri[1, 2].

The fruits are used in medicine and as a condiment. Fruits ovate or broadly elliptical 10-13 mm long narrow winged, reddish yellow have powerful lemon like odour. Fruits yield small quantity of light yellow essential oil having a strong odour of carrot oil[1-3].

Fruits possess diuretic, emmenagogue, aphrodisiac, anthelmintic, antiflatulent, deobstruent, diuretic, anti-inflammatory, antidote, lithontriptic, diaphoretic, stimulent and carminative properties[2,4-11]. *Peucedanum grande* have been reported to have different active constituents including coumarins, 5-geranoxypsoralen, Osthol, camphene, Bergapten, Imperatorin, Isopimperatorin, Hercalenin, Geraniolorin, Columbianadin, Alloimperatorin, 5-methoxy- 8-Hydroxy- psoralen, Byakangelicin and essential oils[3, 12, 13]

The lack of proper production, supply system and the increasing demands of herbal drugs are the major factors promoting the practices of adulteration and substitution. Therefore, standardization of herbal drugs is essential for assuring the therapeutic efficacy of drugs. The present investigation was carried out to develop quality standards and phytochemical analysis of *Peucedanum grande*.

MATERIALS AND METHODS

Collection and Authentication of the Plant material

The seeds of *Peucedanum grande* were purchased from Khari Baoli, local market of Delhi and authenticated by Dr. H. B. Singh National Institute of Science Communication and Information Resources (CSIR), however a voucher submitted in the herbarium of NISCARE, New Delhi (NISCAIR/RHM/f 3/2004/consult/486/62).

Macroscopical and microscopical study

Macroscopical and microscopical characters of the drugs were studied according to the WHO and pharmacopoeial guidelines[14, 15].

Physico-chemical studies

Different physicochemical values such as extractive values (cold & hot extracts), ash values (total ash, acid-insoluble ash & water soluble ash), loss on drying, and pH of 1% and 10%

solution of *P. grande* were determined according to the standard methods[14]. The presence of heavy metals was determined to ensure the safety of the drug in human beings.

Preliminary Phytochemical Analysis

The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents as per method described by Trease and Evans[16]. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins & amino acids, and lipids/fats.

Fluorescence Analysis

Chemical tests of powder drug with different reagents were performed to observe the colour reactions according to the reported method[17].

Determination of total phenolic content[18]

Preparation of standard curve

Standard Gallic acid (10 mg) was dissolved in 100 mL distilled water in a volumetric flask (100 µg/mL of stock solution). From the above stock solution 0.5 to 2.5 ml of aliquots was pipetted out into 25 mL volumetric flasks. Then 10 mL of distilled water and 1.5 mL of Folin-Ciocalteu reagent, diluted according to the label specification to each of the above volumetric flasks were added. After 5 min, 4 mL of 1M sodium carbonate was added and volume was made up to 25 mL with distilled water. After 30 min, absorbance at 765 nm was recorded and calibration curve of absorbance vs concentration was plotted.

Preparation of test sample

Plant material was dried at room temperature and grounded in a mortar. Powder (50 gm) was extracted with 500 mL of methanol by maceration (48 h). The solvent was removed under vacuum and the extract was freeze-dried. From the above prepared test solutions, 1 mL of solution was pipetted out into a 25 mL volumetric flask and then the same steps were followed as given above (standard curve preparation) for color development. The amounts of total phenolics using the standard curve of Gallic acid were determined. (McDonalds) Total phenol values are expressed in terms of gallic acid equivalent (mg/g).

Determination of total flavonoid content[19]

Preparation of standard curve

Aluminum chloride colorimetric method was used for flavonoids determination. Plant extract (0.5 mL of 1:10 mg/mL) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M sodium acetate and 2.8 mL of distilled water. It was kept

at room temperature for 30 min and absorbance of the reaction mixture was measured at 415 nm with a double beam UV spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 10, 20, 40, 50 to 100 µg/mL in methanol.

Preparation of test sample

Plant materials was dried at room temperature and ground in a mortar. Plant powder (50 gm) was extracted with 500 mL of methanol by maceration (48 h). The solvent was removed under the vacuum and the extract was freeze-dried. From the above prepared test solutions, 1 mL of solution was pipetted out into a 25 mL volumetric flask and then the same steps were followed as given above (preparation standard curve) for the colour development.

Development of chromatographic HPTLC fingerprint profile of different extracts

The plant material was coarsely powered and extracted in Soxhlet apparatus for 6-24 h using solvents; petroleum ether, chloroform, and methanol. The extracts were evaporated to dryness in a rota-vapour and the solvents were recovered. Gummy residues so obtained, were stored in deep freezer at -20 °C till further application. TLC and HPTLC samples were prepared by dissolving each extract in their respective solvent to get the concentration; 10 µg/mL. These solutions were further passed through syringe filter to remove any impurities and applied on TLC plate for finger printing analysis. The extract was applied on TLC aluminum sheets silica gel 60 F 254 (Merck) 10 µL each with band length 6 mm using Linomat 5 sample applicator set at a speed of 100 nL/sec CAMAG, Switzerland.

Different solvent systems were used for separation of constituents of different extracts. The chromatograms were developed in twin trough chamber for 20 min up to the distance of 80 mm and the spots were visible without derivatization at 254 and 366 nm wavelengths.

RESULT AND DISCUSSION

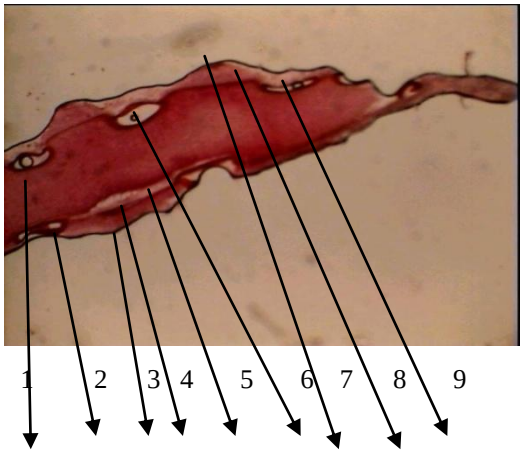
The following results have been obtained under different headings:

Macroscopical characters

Macroscopical characters of fruits mericarp, ovate or broadly elliptical 10-13 mm long; narrowly winged reddish yellow. The fruits have a powerful lemon like odor, irritant and bitter in taste. The glabrous dorsal surface shows three prominent ridges and two lateral wings.

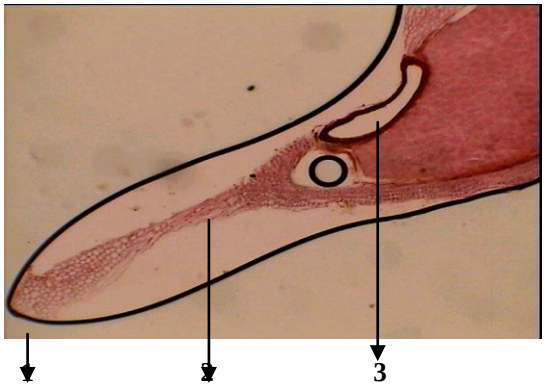
Microscopical characters

Each mericarp has four vittae on dorsal surface and two vittae on commissural surface. The outer epidermis has prominent cuticle and the mesocarp contains lignified reticulate parenchyma below each ridges. The endosperm is much flattened cells. The cells of the embryo are smaller in



1. Mericarp; 2. Commisural vitta; 3. Commisural surface of mericarp; 4. Raphe; 5. Embryo; 6. Dorsal vitta; 7. Vascular Bundle; 8. Endocarp; 9. Cuticle.

Figure 1: T. S. of Mericarp of *P. grande*



1. Cuticle; 2. Vascular bundle; 3. Vittae.

Figure 2: T. S. of Highlighted view of lateral wing

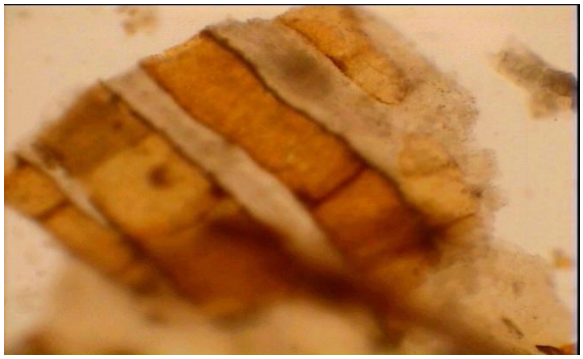


Figure 3: Mesocarp showing fusiform sclereids and fibro vascular tissues associated with vittae.

Figure 4: Fusiform scleried of Mesocarp in surface view.

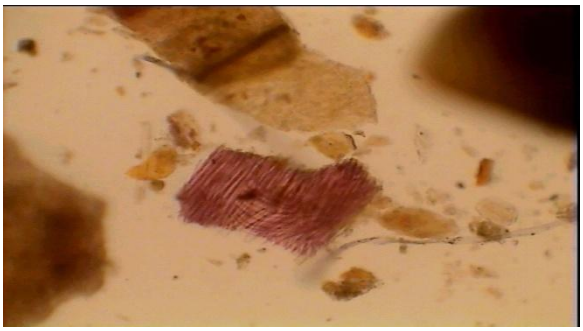


Figure 5: Vascular tissue and associated reticulate Parenchyma of Mesocarp.

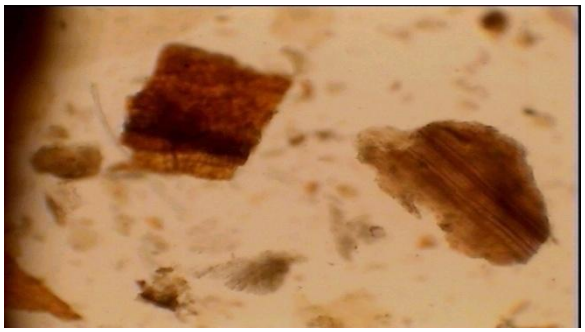


Figure 6: Longitudinal Reticulated Parenchyma.

size as compared to endospermic cells. Prominent raphe is present in the embryo. The testa consists of parenchymatous cells containing fixed oils and aleurone grains. The microscopic characters generally resemble the structure of an umbelliferous fruits having tubular epicarp followed by fibrous mesocarp in the ridge region and endocarp which shows a parquety arrangement in surface view (**Figure 1-6**).

Powder study of *Peucedanum grande*

The epicarp composed of a layer of fairly well defined, colourless cells with uniform well marked cuticular striations; in surface view the cells are variable in shape with thin walls.

The brown fragments of the vittae which are not very numerous, composed of thin walled cells, polygonal in surface view sclereids of the mesocarp, these occurs in groups composed of fairly thick walled cells surface to rectangular in outline with numerous small and conspicuous pits, they are fragment found associated with epicarp.

The occasional groups of reticulate parenchyma of the mesocarp composed of elongated cells with fairly thick, lignified walls. The fragments of fibro-vascular tissues composed of small groups of fibers and vessels showing spiral and annular thickening.

Physico-chemical studies

Different physicochemical parameters such as extractive values (cold, hot & successive), ash values (total ash, acid-insoluble ash & water soluble ash), loss on drying, pH of 1% and 10% solution, and heavy metals of *P. grande* were determined. All the heavy metals (As, Hg, Pd & Cd) were found within the permissible limits as prescribed by the WHO. The results are presented in **Table 1**.

Preliminary Phytochemical Analysis

The preliminary phytochemical screening was carried out using the extracts for presence of different types of chemical constituents. The extracts were analyzed for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins & amino acids, and lipids/fats. Presence and absence of different phyto-constituents are presented in Table 2.

Fluorescence Analysis

Chemical tests of powdered crude drug with different reagents (distilled water, NaOH, HNO_3, H_2SO_4, I_2, HCl, $FeCl_3$, ammonia, glacial acetic acid & picric acid) were studied in day light, U.V. 254 nm and U.V. 366 nm. The results are presented in Table 3.

Total Phenolic content:

Total phenolic content was measured by Folin Ciocalteu method in term of Gallic acid equivalent in mg/g of the extract. The amount of phenolic content was calculated with the help of standard calibration curve (figure 7) and was found to be 146 µg/mL. Results are presented in Table 4.

Table 1: Results of Physico-chemical analysis.

S. No.	Parameter	Value
1.	**Extractive Values (Cold extract)**	**% Extractable matter**
(i)	Petroleum ether extract	7.4
(ii)	Chloroform extract	4.50
(iii)	Methanol cold extract	8.4
(iv)	Aqueous extract	11.5
2.	**Extractive Values (Hot extract)**	**% Extractable matter**
(i)	Petroleum ether extract	9.3
(ii)	Chloroform extract	14.5
(iii)	Methanol extract	12.4
(iv)	Aqueous extract	26.6
3.	**Successive extraction**	**% Extractable matter**
(i)	Petroleum ether extract	9.3
(ii)	Chloroform extract	9.8
(iii)	Methanol extract	3.4
(iv)	Aqueous extract	3.8
4.	**Ash value**	**% Total ash**
(i)	Total ash	8.7
(ii)	Acid insoluble ash	2.5
(iii)	Water soluble ash	5.2
5.	**Loss on drying in crude drug (%)**	6.8
6.	**pH of the drug 1%**	7.34
7.	**pH of the drug 10%**	7.38
8.	**Heavy Metals**	**Value in ppm**
(i)	Arsenic (As)	Less than 0.1 ppm
(ii)	Mercury (Hg)	Less than 0.1 ppm
(iii)	Lead (Pd)	Less than 1.0 ppm
(iv)	Cadmium (Cd)	Less than 0.2 ppm

Table 2: Results of preliminary phytochemical analysis.

Constituent	Extract			
	Petroleum ether	**Chloroform**	**Methanol**	**Aqueous**
Alkaloids	Absent	Absent	Absent	Absent
Carbohydrates	Absent	Absent	Absent	Present
Phenolic compounds	Absent	Present	Present	Present
Flavonoids	Absent	Present	Present	Present
Proteins and amino-acids	Absent	Absent	Present	Present
Lipids / Fats	Present	Present	Absent	Absent

Table 3: Results of fluorescence analysis of crude drug powder.

S. No.	Treatment	Day light	UV light (254 nm)	UV light (366 nm)
1.	Powder as such	Brown	Greenish yellow	Dark greenish yellow
2.	Distilled water	Light brown	Greenish brown	Green
3.	1N NaOH	Gray	Brown	Green
5.	HNO_3	Yellowish green	Yellowish brown	Light greenish yellow
6.	H_2SO_4	Blackish Brown	Blackish black	Reddish Brown
7.	Iodine	Light yellow	Light brown	Greenish brown
8.	Conc. HCl	Brown	Greenish brown	Light green
9.	Ferric chloride	Light green	Light brown	Light green
10.	Ammonia	Light brown	Light greenish yellow	Light greenish yellow
11.	Glacial acetic acid	Yellowish brown	Light yellow	Brown
12.	Picric acid	Light brown	Light green	Dark green

Table 4: Phenolic content of *P. grande*.

S. No.	Concentration of the standard solution (µg/mL)	Absorbance (765 nm)
1.	25	0.086
2.	50	0.200
3.	75	0.408
4.	150	0.796
5.	200	0.966
6.	250	1.265
7.	300	1.412
8.	**Sample** (Fruit)	0.765

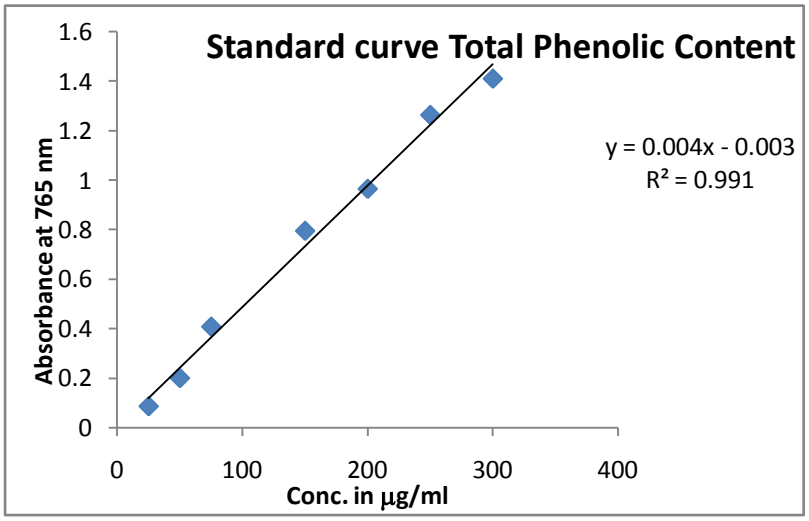


Figure 7: Standard calibration curve for determination of total phenolic content.

Total Flavonoid content

The results of total flavonoids content of *P. grande*, determined by aluminum chloride colorimetric method, are showed in Table 5. The amount of phenolic content was calculated with the help of standard calibration curve (figure 8) and was found to be 91.6 µg/mL.

Chromatographic fingerprint profile of different extracts of Rheum emodi by HPTLC. HPTLC analysis was also performed of methanolic, extracts of *P. grande* for the development of characteristic fingerprint profile, which may be used as markers for quality evaluation and standardization of the drug. The chromatograms obtained after development in different solvent systems followed by scanning at 254 (Table 6) and 366 nm (Table 7) in absorbance mode showed presence of a number of substances in the extracts.

Table 5: Total flavonoids content of *P. grande.*

S. No.	Concentration of the Standard solution (µg/mL)	Absorbance (415 nm)
1.	10	0.055
2.	20	0.109
3.	40	0.232
4.	50	0.293
5.	100	0.591
6	**Sample** (fruit)	0.458

Table 6: Fingerprint of different extracts at 254 nm *Peucedanum grande.*

S. No.	Extract	No. of peaks observed (R_f values) in different solvent systems				
		Petroleum ether: acetone (8:2)	**Ethyl acetate: Ethyl methyl ketone: Formic Acid: Water (5:3:1:1)**	**Benzene: Petroleum Ether: Formic Acid (36:9:5)**	**Chloroform: Methanol: Water (5:4:1)**	**Toluene: Ethyl acetate: Formic Acid (5:4:1)**
1.	Petroleum ether	**03** (0.02, 0.18, 0.27)	**15** (0.06, 0.13, 0.22, 0.27, 0.28, 0.31, 0.33, 0.37, 0.40, 0.44, 0.47, 0.54, 0.77, 0.79, 0.86)	**06** (0.02, 0.11, 0.16, 0.24, 0.40, 0.51)	**12** (0.01, 0.04, 0.33, 0.38, 0.39, 0.45, 0.56, 0.65, 0.67, 0.73, 0.74, 0.93)	**07** (0.03, 0.39, 0.53, 0.59, 0.63, 0.66, 0.79)
2.	Chloroform	**01** (0.01)	**02** (0.79, 0.84)	**08** (0.02, 0.10, 0.13, 0.20, 0.25, 0.33, 0.43, 0.49)	**03** (0.03, 0.90, 0.95)	**08** (0.02, 0.41, 0.50, 0.54, 0.60, 0.65, 0.70, 0.76)
3.	Acetone	**05** (0.02, 0.05, 0.12, 0.16, 0.25)	**04** (0.42, 0.57, 0.73,0.82)	**06** (0.03, 0.11, 0.14, 0.20, 0.34, 0.42)	**08** (0.02, 0.03, 0.27, 0.29, 0.38, 0.70, 0.72, 0.96)	**10** (0.03, 0.10, 0.18, 0.30, 0.39, 0.48, 0.51, 0.57, 0.61, 0.66)
4.	Methanolic	-	**04** (0.59, 0.65, 0.77, 0.82)	**03** (0.02, 0.09, 0.42)	**03** (0.82, 0.88, 0.97)	**09** (0.09, 0.40, 0.48, 0.51, 0.55, 0.57, 0.59, 0.65, 0.74)
5.	Water	-	**01** (0.83)	-	**02** (0.01, 0.98)	**02** (0.01, 0.94)

Table 7: Fingerprint of different extracts at 366 nm

S. No.	Extract	No. of peaks observed (Rf values) in different solvent systems				
		Petroleum ether: acetone (8:2)	**Ethyl acetate : Ethyl methyl ketone :Formic Acid :Water (5:3:1:1)**	**Benzene: Petroleum Ether: Formic Acid (36:9:5)**	**Chloroform : Methanol: Water (5:4:1)**	**Toluene: Ethyl acetate :Formic Acid (5:4:1)**
1	Petroleum ether	**03** (0.02, 0.17, 0.27)	**03** (0.06, 0.78, 0.86)	**06** (0.01, 0.11, 0.16, 0.24, 0.40, 0.50)	**07** (0.08, 0.33, 0.35, 0.37, 0.40, 0.74, 0.93)	**07** (0.03, 0.32, 0.39, 0.52, 0.58, 0.64, 0.80)
2	Chloroform	**01** (0.01)	**05** (0.04, 0.16, 0.22, 0.79, 0.84)	**08** (0.02, 0.09, 0.13, 0.20, 0.25, 0.33, 0.42, 0.49)	**02** (0.03, 0.90)	**10** (0.02, 0.33, 0.41, 0.50, 0.54, 0.60, 0.65, 0.70, 0.76, 0.91)
3	Acetone	**04** (0.02, 0.05, 0.16, 0.25)	**01** (0.73)	**06** (0.03, 0.11, 0.14, 0.20, 0.34, 0.42)	**08** (0.01, 0.03, 0.14, 0.18, 0.26, 0.29, 0.69, 0.95)	**09** (0.02, 0.10, 0.18, 0.31, 0.38, 0.50, 0.56, 0.61, 0.71)
4	Methanolic	-	**03** (0.53, 0.58,0.78)	**03** (0.01, 0.09, 0.42)	**01** (0.88)	**06** (0.22, 0.40, 0.51, 0.57, 0.65, 0.74)
5	Water	-	**04** (0.05, 0.16, 0.29, 0.50)	-	**01** (0.01)	**04** (0.01, 0.39, 0.64, 0.84)

Figure 9 & 10 show the 3D view of different extract of *P. grande* with Petroleum ether: Acetone & with Toluene: Ethyl acetate: Formic acid at 254 nm, respectively. Figure 11 & 12 show the 3D view of different extract of *P. grande* with Petroleum ether: Acetone & with Toluene: Ethyl acetate: Formic acid at 366 nm, respectively. HPTLC chromatograms of methanolic extracts of *P. grande* at 254 and 366 nm in solvent systems Petroleum ether: Acetone (8:2) & in Toluene: Ethyl acetate: Formic acid (5:4:1) are presented as figure 13 & 14, respectively.

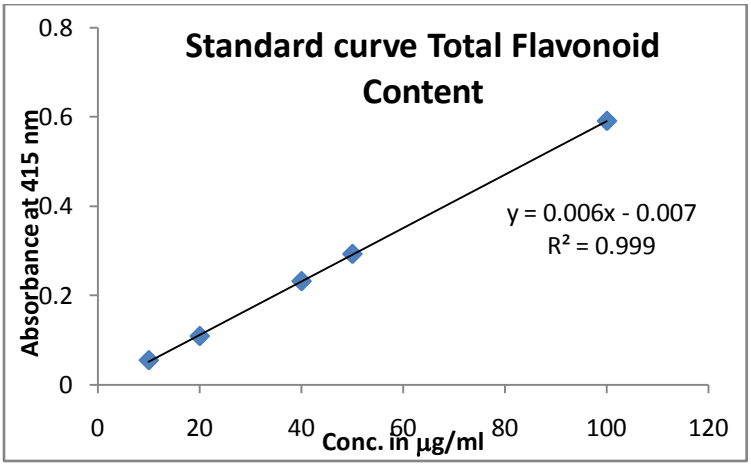


Figure 8: Standard calibration curve for determination of total flavonoid content.

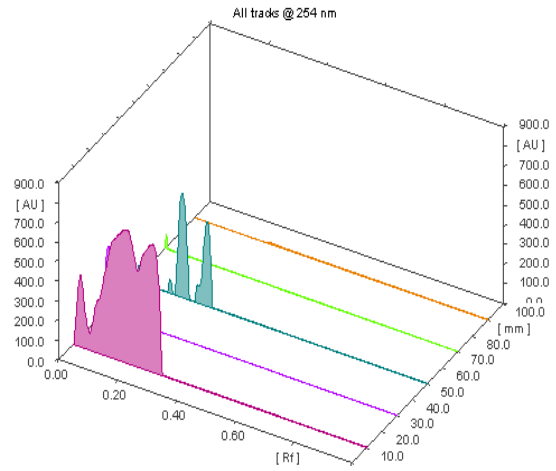


Figure 9: **3D view of different extract of *P. grande* with solvent system Petroleum ether: Acetone (8:2) at 254 nm.**

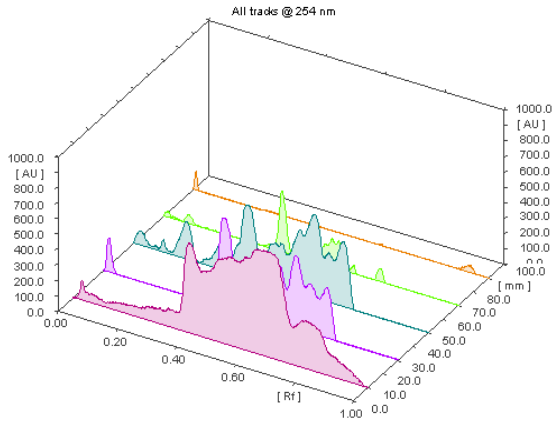


Figure 10: **3D view of different extract of *P. grande* with solvent system Toluene: Ethyl acetate: Formic acid at 254 nm.**

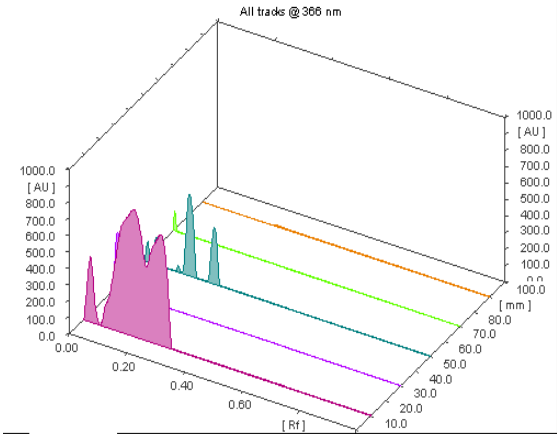


Figure 11: **3D view of different extract of *P. grande* with solvent system Petroleum ether: Acetone (8:2) at 366 nm.**

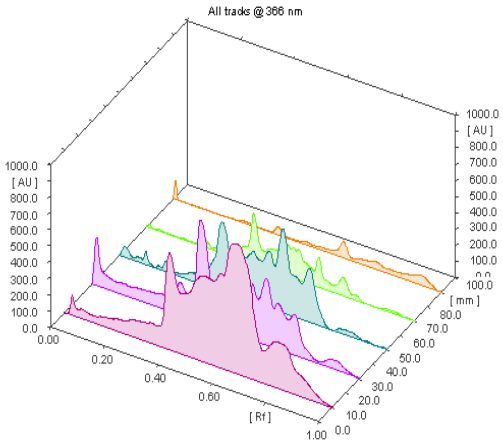


Figure 12: 3D view of different extract of *P. grande* with solvent system Toluene: Ethyl acetate: Formic acid at 366 nm.

This study on the development of quality standards and phytochemical analysis of *Peucedanum grande*, revealed a set of parameters which may enable to those who handle this plant to maintain its quality control. Adulteration and substitution have become a major problem due to absence of standards relating to genuineness of drug. The quality of natural medicines must be as high as that of other synthetic medicinal preparations. Quality refers to intrinsic value of the drug, the amount of medicinal principles or active constituents present.

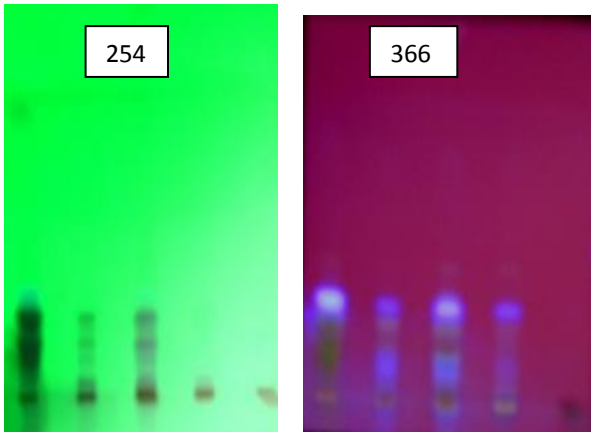


Figure 13: HPTLC chromatograms of methanolic extracts of *P. grande* at 254 and 366 nm in solvent system Petroleum ether: Acetone (8:2).

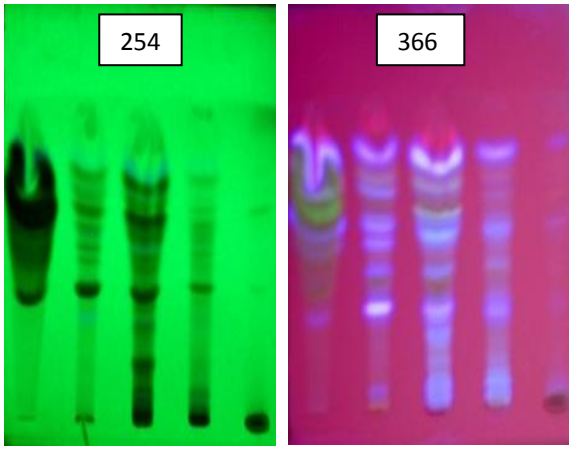


Figure 14: HPTLC chromatograms of methanolic extracts of *P. grande* at 254 and 366 nm in solvent system Toluene: Ethyl acetate: Formic acid (5:4:1).

The pharmacognostical parameters including HPTLC are helpful for the future identification and authentification of this plant in the herbal industry. The physical parameters, such as loss on drying, ash values and extractive values will be helpful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in future. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. The plant may be considered as biosynthetic laboratory for a variety of compounds (secondary metabolites) like alkaloids, glycosides, flavonoids, volatile oils, and saponins that exert physiological effects. The curative properties of medicinal plants are due to the presence of various secondary metabolites. Thus the preliminary screening tests may be useful in the detection of bioactive principles. HPTLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. More phytochemical research work is required for isolation, purification and characterization of biologically compounds.

CONCLUSION:

Standardization of herbal drugs is a topic of great concern. They are subject to variability as derived from heterogeneous sources. This variability can have both merits and demerits. The main demerits are that the activity of the material may vary and that inferior material may be produced. *Peucedanum grande* is an important plant and has been found to have various

biological properties. So efforts have been made to provide scientific data to standardize the plant material for further studies. Microscopic, macroscopic data and other physical values including HPTLC will help to identify the correct species of the plant. The research out comings of the standardization parameters can also be used for evaluating the quality and purity of the drug and its formulation.

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