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Preparation of Tuvaraka Taila by classical method and quality specifications thereon

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Abstract

Seed oil extracted from matured fruits of Hydnocarpus pentandra known as Tuvaraka taila is one among the most trusted Ayurvedic treatment for all types of kushtha roga (skin diseases). The oil also known as chaulmoogra oil is a potential healer in modern therapeutics also. H. pentandra is an evergreen deciduous tree found in the Western Ghats. Extraction of the oil from dry seeds of Tuvaraka has been well documented in classical references. Proper extraction of oil from the seeds is essential to promote its therapeutic use. In this article, standard operating procedure for extraction of Tuvaraka taila and its chemical analysis as per prescribed analytical parameters is reported with suitable discussion. The method of extraction of oil in comination with the chemical analyses thereof will serve as quality control parameters to conclude standard operating procedures for Tuvaraka taila of Ayurveda.

Keywords: Analytical standardization, Chaulmoogra oil, Hydnocarpus pentandra, standard operating procedure, skin diseases, Tuvaraka.

Introduction

Tuvaraka (*Hydnocarpus pentandra* Buch.-Ham.) is one among the most trusted drugs from the treasure of Ayurveda. The oil extracted from the seeds of '*pakwatuvarakaphala'*, commonly known as 'chaulmoogra oil' is mentioned as a potential healer for all types of kushtha roga - a group of skin diseases[1]. *Tuvaraka*is an evergreen deciduous tree, widely grown in the Western Ghats up to 15 meters or more in height[1]. Its well grown fruits will be globose, mammilate and tomentose with 5 to 10 cm in diameter. The seeds will be 15 to 20 in a fruit with striate, sub ovoid and obtusely angular measuring 2 to 2.5 cm long. The ripe fruits collection is usually done by the end of summer season i.e. before the rainy season begins[1]. The March and April months are the ideal time for collection of ripe fruits. Once when the fruits are collected the seeds inside are separated carefully, spread in clean wider stainless steel trays and dried under hot sun. This dry seeds are now subjected for extraction of oil. For this we find well documented classical methods of oil extraction[1].In this article classical method of extraction of *Tuvaraka beeja taila* is elaborately studied and discussed along with its analytical study through organoleptic characters, physico-chemical examination and HPTLC fingerprinting.

Materials and methods

Collection

The mature fruits of *Tuvaraka* (12 kg) were collected from two well grown trees in the herbal garden *Rajavana* of S. D. M. College of Ayurveda, Kuthpady, Udupi. Oil extraction from seeds was done in S. D. M. Ayurveda Pharmacy, Kuthpady, Udupi. The fruits were gently pounded and the dry good quality seeds inside were separated (8 kg). These seeds were evenly spread in two steel trays and dried daily under hot sun. After two weeks, the thin layer of wet pulp around the seeds was completely dry emitting pleasant characteristic fragrance.

Extraction of oil

Eight kilograms of seedswere finely pounded in a pulveriser. The snigdha curna (fine powder) obtained was fed to the grinder with sufficient quantity so as to render paste form to the fine powder. This paste (8.5 kg) was transferred to a wide mouthed iron *kadhai* (vessel) and placed over mild fire adding 34 liters (4 times of the drug) of water. The boiling was carried out with frequent stirring, approximately after two hours; the oilstarted appearing on the surface of the liquid in globular form. When boiled further the oil globules unite together to form an oil layer over the liquid. The process of boiling is continued further until an appreciable layer of oil is seen on the surface of the liquid. Later the fire was put off and the liquid was allowed to become stable. The supernatant oil layer was carefully skimmed off

and collected in another stainless steel vessel. The remaining liquid was further boiled and the supernatant oil layer over the liquid surface was collected for 3 times. It was packed in air tight bottles with neat labeling and preserved in dark place until analysis.

Analytical study

It was performed in Pharmaceutical Chemistry wing of S. D. M. Centre for Research in Ayurveda and Allied Sciences, Udupi. Organoleptic characteristics like colour, taste, odour and consistency; physico-chemical parameter like loss on drying, refractive index, acid value, saponification value, iodine value, specific gravity and viscosity[2], and identity test by HPTLC[3,4] was recorded.

HPTLC

Sample obtained by the procedure for the determination of unsaponifiable matter is dissolved in 10 ml of chloroform. Four, 8 and 12 µl of the above was applied using CAMAG Linomat5 applicator, separation was obtained using toluene: ethyl acetate (9: 1) as mobile phase on silica gel G F254 pre-coated on aluminium sheets of 0.2 mm thickness[3,4]. The R_f values were determined from the photo-documentation performed using CAMAG photo-documentation cabinet and the plates were scanned under 254 nm, 366 nm and 620 nm after derivatisation using CAMAG Scanner 4.

Results and Discussion

The oil obtained from 1st, 2nd and 3rd supernatant oil layer was 510, 260 and 200 ml respectively (yield 970 ml). The oil was a pale yellow viscous liquid with greasy touch and characteristic odour (Figure 1). Loss on drying indicates the amount of volatile matter including moisture; 37.18% was the result for the sample. pH is the scale that represents the relative acidity or the alkalinity of the sample; 5 was the result obtained, which means the oil is of acidic nature.Viscosity is index of resistance offered by the surface to flow of a liquid; higher the viscosity of a liquid, the greater is the resistance to flow, if viscosity of the oil preparation is increases, the rate of absorption decreases. If the oil is less viscous this means rate of absorption is very high; the oil is better absorbed into the skin. Viscosity of the oil is found to be 112 cp. Refractive index indicates density of sample compared to air and liquid media; the value for *tuvaraka taila* was found to be 1.47583. Specific gravity indicates the weight of a liquid, compared with that of distilled water the value was found to be 0.9452. The amount of alkali needed to saponify a given quantity of oil will depend upon number of COOH group present; the saponification value also indicates the average molecular weight /chain length of all fatty acids present. Longer the chains, fatty acids have low saponification value, and the shorter chain fatty acids have high saponification value. Shorter chain fatty acids (high saponification value) have faster rate of absorption than longer chain fatty acids; saponification value of the sample was found to be 185.15. Unsaponifiable matter indicates components oils other than fatty acids, the value for this sample was found to be 0.76%.The acid valueindicates the presence of free fatty acids in the oil which are responsible of rancidity of the compounds; higher the free fatty acid more is the rancidity. This helps to decide the shelf life of the oil; acid value for *tuvaraka taila* was found to be 6.02. Iodine value indicates the degree of unsaturation of oil; greater the degree of unsaturation, higher will be possibility of absorption and atmospheric oxidation leading to rancidity. More iodine number, the more unsaturated fatty acid bonds are present; unsaturated fatty acid is better absorbed than saturated fatty acids, the value was found to be 9.80 (Table 1). These constants can be used as standard values to derive quality parameters for *tuvaraka taila.*

On photodocumentation at 254 nm *tuvaraka taila* showed 8 spots (Table 2). At 366 nm there were 12 spots (Table 2). Under white light, after derivatisation with vanillin sulphuric acid *tuvaraka taila* showed 10 spots (Table 3). The unsaponifiable matter dissolved in chloroform can be used as sample for fingerprinting of the formulation *tuvaraka taila*.

On densitometric scan at 254 nm *tuvaraka taila* showed 11 peaks, peak with R_f 0.07 being the major spot contributing to 34.21%and R_f 0.65 was the spot that contributed to 19.83%; these two were major spots (Figure 3). At 366 nm *tuvaraka taila* showed 8 peaks, peaks with R_f 0.69 was the major spot of 52.63%. Next highest peak with R_f 0.48 contributes to 31.08 % (Figure 4). HPTLC at both the wavelengths were found to be useful for fingerprinting the unsaponifiable matter of *tuvaraka taila*.

Table 1: Physico-chemical constants of *Tuvaraka taila*

Parameter	*Tuvaraka* taila
LOD	37.18
pH	5
Viscosity at 29^0C	112 cp
Refractive index 29^0C	1.47583
Specific gravity at 29^0C	0.9452
Saponification value	185.15
Acid value	6.02
Iodine value	9.80
Unsaponifiable matter	0.76

Table 2:R_f values of TLC of *Tuvaraka taila*

At 254 nm	At 366 nm	Post derivatisation
-	-	0.06 Blue
0.11 Green	-	-
-	0.13 F blue	0.13 Blue
-	0.16 F blue	-
-	0.18 F blue	0.18 Blue
-	0.22 F blue	-
-	-	0.27 Blue
-	0.32 F blue	-
0.40 Green	-	0.40 Purple
0.44 Green	0.44 F blue	-
-	0.47 F violet	-
-	-	0.52 Blue
0.55 Green	-	-
-	0.58 F blue	0.58 Blue
0.65 Green	-	-
0.72 Green	0.72 F blue	-
-	-	0.74 L blue
0.79 Green	0.79 F blue	-
-	-	0.85 Blue
0.88 Green	-	-
-	0.90 F violet	-
-	-	0.92 L blue
-	0.95 F violet	-
F- Fluorescent		

***Tuvaraka* tree**

Tuvaraka phala

Drying of *tuvaraka beeja*

Weighing of *tuvaraka beeja*

Pounding of *tuvaraka beeja*

Preparation of *tuvaraka beeja kalka*

***Tuvaraka beeja kalka* added with 3 times of water**

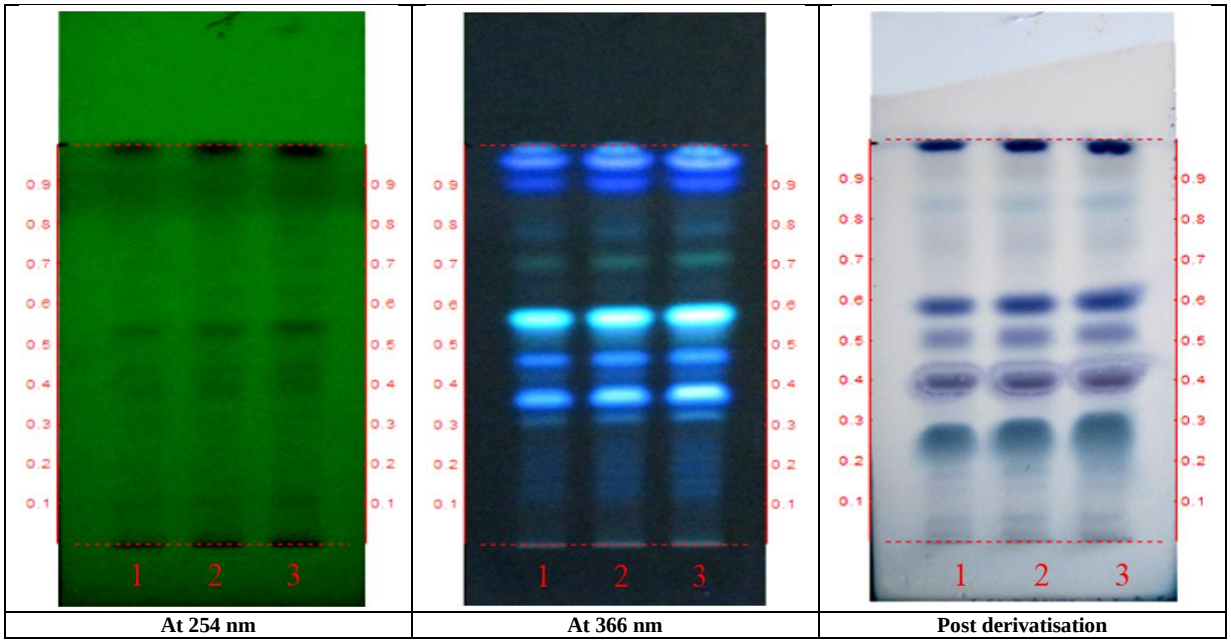
Collection of supernatant oil layer

***Tuvaraka taila paka* from skimmed off supernatant layer**

Collection of *tuvaraka taila*

***Tuvarakataila* packed in dropper bottles for clinical use**

Figure 1: Procedures for preparation of *Tuvaraka taila*

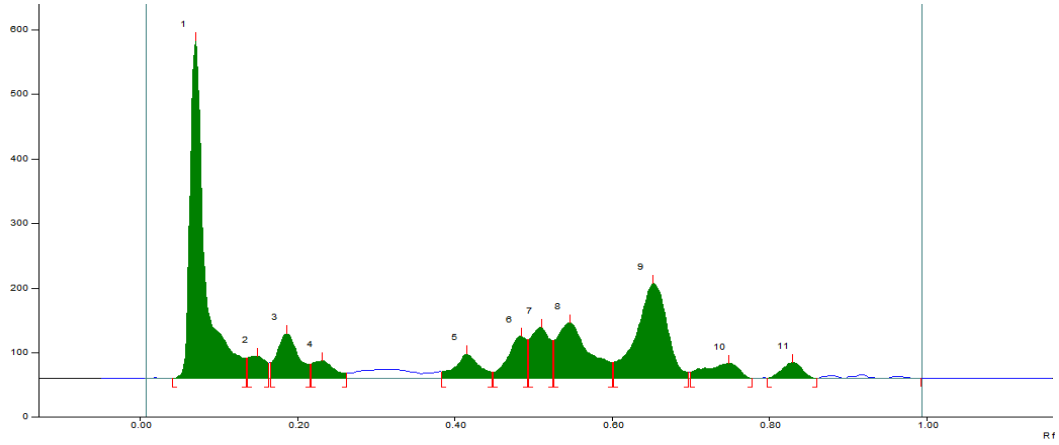


At 254 nm | **At 366 nm** | **Post derivatisation**

Figure 2: TLC photodocumentation of *Tuvaraka taila*

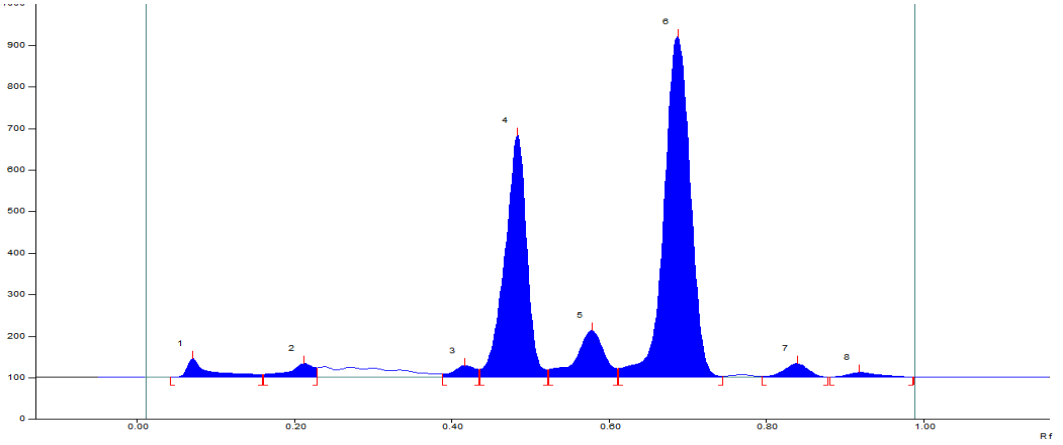
Solvent system – Toluene: Ethyl acetate 9:1

Track 1 – 4 µl; 2 - 8 µl; 3 – 12 µl



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.2 AU	0.07 Rf	522.3 AU	46.81 %	0.14 Rf	31.0 AU	7001.1 AU	34.21 %
2	0.14 Rf	31.3 AU	0.15 Rf	34.4 AU	3.08 %	0.16 Rf	23.7 AU	558.7 AU	2.73 %
3	0.17 Rf	23.9 AU	0.19 Rf	69.4 AU	6.22 %	0.22 Rf	21.4 AU	1339.3 AU	6.55 %
4	0.22 Rf	21.8 AU	0.23 Rf	27.4 AU	2.45 %	0.26 Rf	7.6 AU	543.3 AU	2.66 %
5	0.38 Rf	10.2 AU	0.42 Rf	37.9 AU	3.40 %	0.45 Rf	9.7 AU	823.3 AU	4.02 %
6	0.45 Rf	9.8 AU	0.49 Rf	64.9 AU	5.82 %	0.49 Rf	59.9 AU	1113.2 AU	5.44 %
7	0.50 Rf	60.0 AU	0.51 Rf	78.5 AU	7.03 %	0.53 Rf	58.6 AU	1371.0 AU	6.70 %
8	0.53 Rf	59.0 AU	0.55 Rf	86.4 AU	7.74 %	0.60 Rf	24.4 AU	2478.2 AU	12.11 %
9	0.60 Rf	24.7 AU	0.65 Rf	146.6 AU	13.14 %	0.70 Rf	9.4 AU	4058.2 AU	19.83 %
10	0.70 Rf	9.6 AU	0.75 Rf	23.8 AU	2.14 %	0.78 Rf	0.2 AU	727.1 AU	3.55 %
11	0.80 Rf	0.5 AU	0.83 Rf	24.4 AU	2.18 %	0.86 Rf	0.1 AU	448.8 AU	2.19 %

Figure 3: HPTLC densitometric scan of *Tuvaraka taila* at 254 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.1 AU	0.07 Rf	44.7 AU	2.69 %	0.16 Rf	6.6 AU	893.2 AU	2.42 %
2	0.16 Rf	6.6 AU	0.21 Rf	32.3 AU	1.95 %	0.23 Rf	22.3 AU	734.4 AU	1.99 %
3	0.39 Rf	7.7 AU	0.42 Rf	27.8 AU	1.67 %	0.44 Rf	18.4 AU	544.6 AU	1.47 %
4	0.44 Rf	18.8 AU	0.48 Rf	581.7 AU	35.01 %	0.52 Rf	18.1 AU	11484.3 AU	31.08 %
5	0.52 Rf	18.4 AU	0.58 Rf	112.2 AU	6.75 %	0.61 Rf	20.4 AU	2822.7 AU	7.64 %
6	0.61 Rf	20.5 AU	0.69 Rf	819.8 AU	49.34 %	0.75 Rf	2.0 AU	19445.5 AU	52.63 %
7	0.80 Rf	1.6 AU	0.84 Rf	32.1 AU	1.93 %	0.88 Rf	0.0 AU	711.8 AU	1.93 %
8	0.88 Rf	0.0 AU	0.92 Rf	11.0 AU	0.66 %	0.99 Rf	0.1 AU	309.5 AU	0.84 %

Figure 4: HPTLC densitometric scan of *Tuvaraka taila* at 366 nm

Conclusion

Tuvaraka taila is said to be the best *kushtaghna taila*. All the details of drug collection and oil extraction from the seeds of ripe *tuvaraka* fruit is well explained in *Sushruta samhita*[1]. With that classical reference in backdrop, *tuvaraka taila* was extracted as per the advised standard operating procedures (SOP) and subjected for different analysis thereon. The result of analytical study with TLC and HPTLC fingerprints can be used as quality test to identify and check quality of *tuvaraka taila* prepared as per classical text.

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