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Chemo-profiling of *Pandanus odoratissimus* L. fragrant inflorescence used in ayurveda, through HPTLC and GC

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Abstract
About: Herbal material used in therapeutics contains a lot of chemical constituents which can be classified primarily as primary and secondary metabolites. Chemical evaluation of herbal material involves different chemical tests, assays, isolation, purification and there by identification of active constituents. *Pandanus odoratissimus* L. commonly known as *Ketaki*, the essential oil extracted out of the flowers beneficial in arthritis, antispasmodic and nootropic effect.
Materials and Methods: Inflorescences of test drug separated from their colourful bracts shade dried for a day and cut in to small piece. For HPTLC n-hexane extract of this sample was applied on a precoated aluminium plates using Benzene as mobile phase. Developed plates were visualized in UV 254, 366, under white light and then derivatised with vanillin sulphuric acid and scanned under UV 254 and 366 nm. Methylated volatile oil of test sample was introduced into a stream of helium, for Gas Chromatography study. The results were tabulated as per the retention time.
Results: HPTLC photo documentation of n-hexane extract of drug has shown 5 peaks at 254nm, whereas 6 peaks at 366nm. After post derivatisation at 620nm drug has shown 9 peaks. GC-MS analysis of volatile oil of test drug showed presence of totally 39 compounds among which 32 were identified by their Mass spectra with MS-Library.

Keywords: *Pandanus odoratissimus* L., GC-MS analysis, HPTLC

1. Introduction
Traditional medicine is attracting health care system recently through its natural products. Historically alternative, complementary, non-conventional are terms used to denote this system [1]. Traditional medicine uses various plant, animal or mineral products; based on their own theory, spiritual therapy to cure illness and to maintain health[2]. Ayurveda, Indian system of medicine generally used health care system now attaining popularity worldwide. Personalized approach, use of natural products which are easily available and lesser side effects are prime features of Ayurveda [3]. Currently revival of interest in natural products resulted in its question related to efficacy, safety and evident activity profile. Hence everyone interested to generate standard quality control parameters of raw material [4]. Herbal drug standardization guidelines start from authentication, macroscopy, microscopy, powder identity, isolation techniques like TLC, HPTLC, and GC etc [5].
In most of alternative therapies raw drugs are used as whole than in extract or molecular form [6]. Herbal material used in therapeutics contains a lot of chemical constituents which can be classified primarily as primary and secondary metabolites. These secondary metabolites may be tannin, alkaloid, glucoside, volatile oil, flavonoid, saponin, terpenes [7]. Chemical evaluation of herbal material involves different chemical tests, assays, isolation, purification and there by identification of active constituents [8].
Chromatographic techniques are primitive, physical methods of separation in which compounds to be separated are distributed between two phases. One of which is stationary phase and other a mobile phase. Basically, chromatographic techniques like HPTLC, HPLC, and GLC give out chromatograms which serve as fingerprint. This fingerprint of a particular part of plant will be same if conditions are maintained. Thus, these are also reproducible anywhere else, provided maturity, geographical condition of herbal material from where it is procured [9]. TLC is used for separation of simple mixture where speed, cost effectiveness

and simplicity are prime factors. HPTLC is an advanced technique with high sample throughput, one of the best methods of standardization of plant drugs. Plants with multiple phytochemical constituents can be easily separated and quantified through this [10]. Gas chromatography is usually used in analysis of compounds that can be vaporized without decomposition. This is similar to column chromatography, but here separation is carried out between a liquid stationary phase and a gas mobile phase. Hence GC is also sometimes known as vapour-phase chromatography or Gas- liquid chromatography [11]. Hence gaseous compounds are analysed, which interact with the walls of column coated with a stationary phase, which cause each molecule to elute at a different time known as retention time. This retention time comparison gives GC its analytical usefulness [12]. *Pandanus odoratissimus* L. commonly known as *Ketaki* is a densely branched shrub; the fragrant spadices which are used in the extraction of Kewda attar, the most popular perfume extracted and used in India since ancient days [13]. The male inflorescences are valued for the fragrant smell emitted by the tender white spathes covering the flowers and valuable attar obtained from them. The essential oil extracted out of the flowers (*Ketaki arka*) effective remedy in headache, rheumatoid arthritis, antispasmodic and nootropic effect [14]. Hence with all these backgrounds it is planned to conduct comparative chemical standardization of Ketaki arka (volatile oil), through HPTLC and GC techniques.

2. Materials and Method

Sample collection

Male Inflorescence of *Pandanus odoratissimus* L. were collected from their natural habitat, photographs were taken, authenticated using floras and botanist's opinion and samples deposited at SDM centre for research in Ayurveda and Allied sciences (Voucher No.12100801). Inflorescences were separated from their colourful bracts shade dried for a day and cut in to small pieces [15].

HPTLC: 1g of test drug powder was extracted with 10 ml of *n*-hexane. 5, 10 and 15 µl of the above extract was applied on a pre-coated silica gel F_{254} on aluminium plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Benzene. The developed plates were visualized in UV 254, 366, under white light and then derivatised with vanillin sulphuric acid and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded [16].

Gas chromatography

Volatile oil in the test drug was estimated by distilling the 500g of coarsely powdered inflorescence of *Pandanus odoratissimus* L. with a mixture of 800ml of water, taken in a round bottom flask and connected to the Clevenger's apparatus and distillation was carried out. Volume of the essential oil was measured and sent for the Gas Chromatography study [9]. Methylated test sample was introduced into a stream of helium. The results were tabulated as per the retention time [17].

3. Results

HPTLC photo documentation of n-hexane extract of *P.odoratissimus* L. has shown 5 peaks at 254nm, whereas 6 peaks at 366nm. After post derivatisation at 620nm drug has shown 9 peaks. (Figure 1, 2, 3, 4 and Table 1).

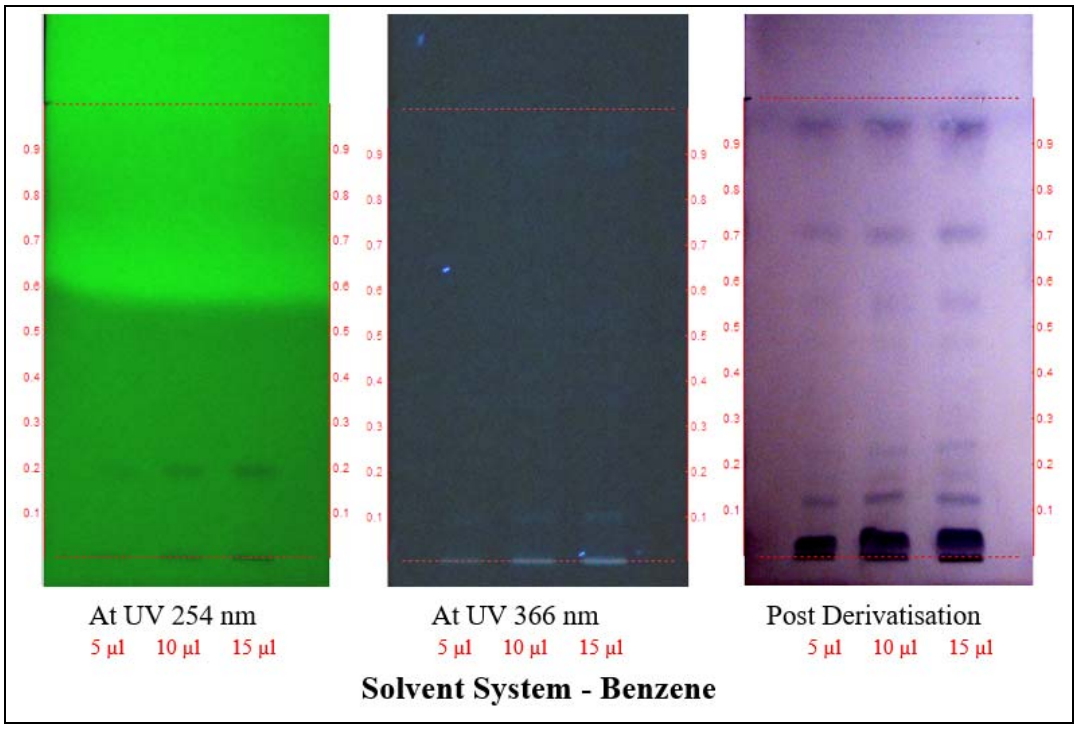
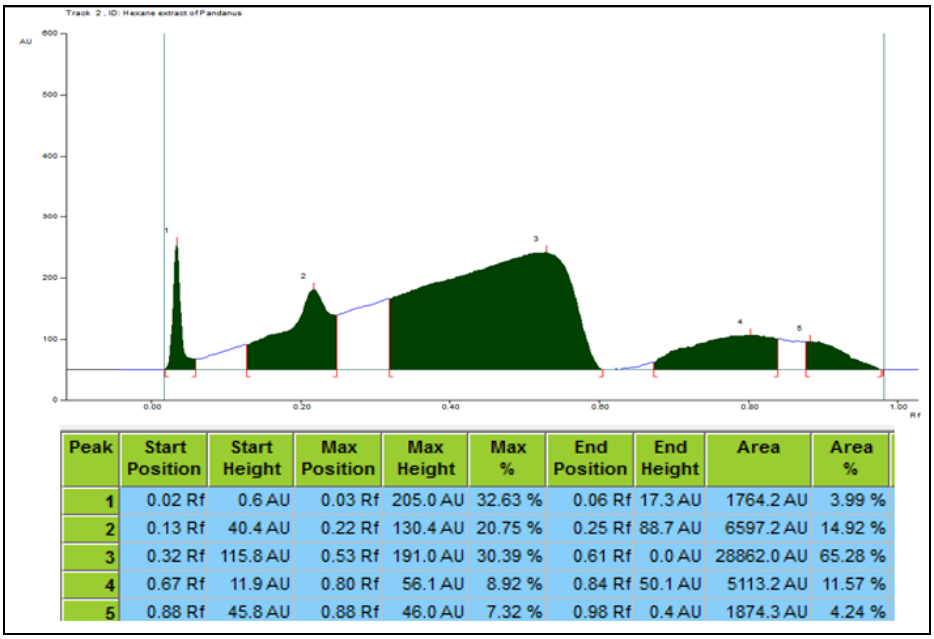


Fig 1: TLC Photodocumentation of *n*-hexane extract of *Pandanus odoratissimus L.*

Table 1: R_f value of *n*- hexane extract of *Pandanus odoratissimus* L.

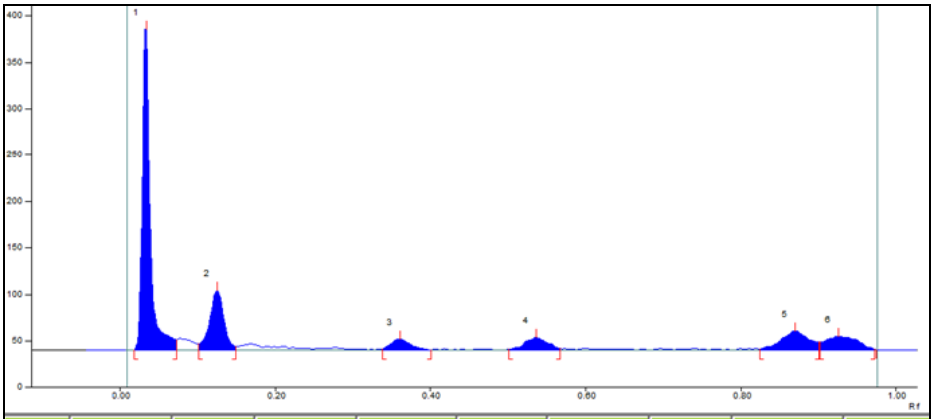
At UV 254 nm	At UV 366 nm	Post –Derivatisation
0.04 L Green	-	0.04 Blue
-	0.10 F Green	-
-	-	0.13 Violet
0.18 Green	-	0.18 Violet
-	--	0.24 Violet
-	0.36 F L Blue	-
-		0.46 Violet
-	0.55 F L Blue	0.55 Violet
-		0.70 Violet
-	0.90 F L Blue	-
-	-	0.94 Blue

D- Dark, L - Light, F- Flourescent



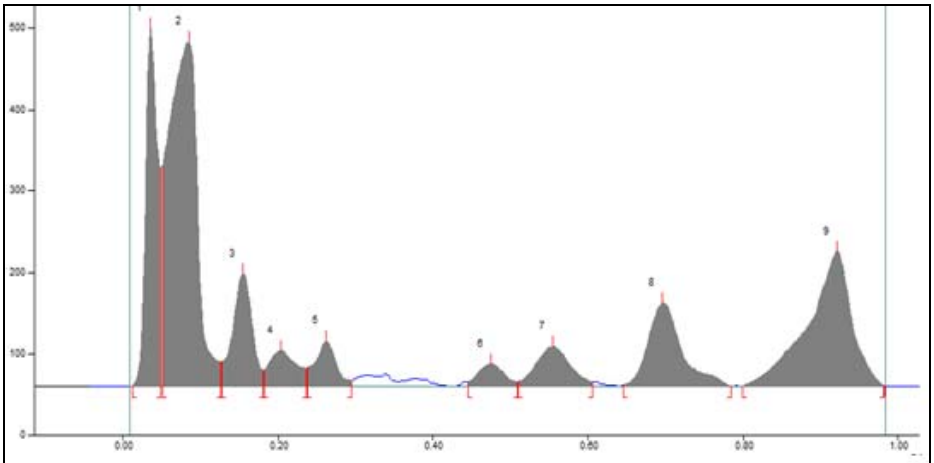
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.6 AU	0.03 Rf	205.0 AU	32.63 %	0.06 Rf	17.3 AU	1764.2 AU	3.99 %
2	0.13 Rf	40.4 AU	0.22 Rf	130.4 AU	20.75 %	0.25 Rf	88.7 AU	6597.2 AU	14.92 %
3	0.32 Rf	115.8 AU	0.53 Rf	191.0 AU	30.39 %	0.61 Rf	0.0 AU	28862.0 AU	65.28 %
4	0.67 Rf	11.9 AU	0.80 Rf	56.1 AU	8.92 %	0.84 Rf	50.1 AU	5113.2 AU	11.57 %
5	0.88 Rf	45.8 AU	0.88 Rf	46.0 AU	7.32 %	0.98 Rf	0.4 AU	1874.3 AU	4.24 %

Fig 2: HPTLC Densitometric scan of *n*- hexane extract *of Pandanus odoratissimus* L. at 254 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.3 AU	0.03 Rf	345.4 AU	73.40 %	0.07 Rf	11.2 AU	2904.3 AU	52.47 %
2	0.10 Rf	6.4 AU	0.13 Rf	64.4 AU	13.69 %	0.15 Rf	4.0 AU	958.5 AU	17.32 %
3	0.34 Rf	1.3 AU	0.36 Rf	11.8 AU	2.50 %	0.40 Rf	0.1 AU	243.5 AU	4.40 %
4	0.50 Rf	0.5 AU	0.54 Rf	13.5 AU	2.87 %	0.57 Rf	1.6 AU	325.4 AU	5.88 %
5	0.83 Rf	1.1 AU	0.87 Rf	20.5 AU	4.36 %	0.90 Rf	8.6 AU	598.8 AU	10.82 %
6	0.90 Rf	8.7 AU	0.93 Rf	14.9 AU	3.17 %	0.97 Rf	0.5 AU	505.0 AU	9.12 %

Fig 3: HPTLC Densitometric scan of *n*- hexane extract of *Pandanus odoratissimus* L. at 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.3 AU	0.04 Rf	440.0 AU	30.49 %	0.05 Rf	66.4 AU	5653.3 AU	15.10 %
2	0.05 Rf	268.6 AU	0.09 Rf	422.9 AU	29.30 %	0.13 Rf	29.7 AU	12957.7 AU	34.62 %
3	0.13 Rf	30.1 AU	0.16 Rf	138.1 AU	9.57 %	0.18 Rf	19.7 AU	2784.1 AU	7.44 %
4	0.18 Rf	19.9 AU	0.20 Rf	43.7 AU	3.03 %	0.24 Rf	22.2 AU	1250.4 AU	3.34 %
5	0.24 Rf	22.6 AU	0.26 Rf	55.5 AU	3.85 %	0.30 Rf	7.1 AU	1207.9 AU	3.23 %
6	0.45 Rf	5.3 AU	0.48 Rf	27.6 AU	1.92 %	0.51 Rf	5.5 AU	748.3 AU	2.00 %
7	0.51 Rf	5.8 AU	0.56 Rf	48.5 AU	3.36 %	0.61 Rf	5.5 AU	1739.2 AU	4.65 %
8	0.65 Rf	1.8 AU	0.70 Rf	101.6 AU	7.04 %	0.79 Rf	0.2 AU	3570.1 AU	9.54 %
9	0.80 Rf	0.1 AU	0.92 Rf	165.2 AU	11.45 %	0.98 Rf	0.8 AU	7518.5 AU	20.09 %

Fig 4: HPTLC Densitometric scan of *n*- hexane extract of *Pandanus odoratissimus* L. at 620 nm after derivatisation

GC-MS analysis of volatile oil of test drug showed presence of totally 39 compounds among Which 32 were identified by their Mass spectra with MS-Library. (Table 2)

Table 2: GC-MS identification of volatile oil content of *Pandanus odoratissimus* L.

No	RT	Probable name of the compound	% area
1	3.08	2,4-dimethyl-Pentane	1.24
2	3.31	2-ethyl- 1Hexanol	0.77
3	3.89	Unidentified	1.92
4	4.17	3-Hexanone	1.06
5	4.59	Tetrachloroethylene	2.58
6	7.05	2-bromo- Hexane	1.70
7	7.21	3-bromo-3-methyl- Pentane	1.82
8	8.50	2-ethyl- 1Hexanol	0.90
9	9.54	1-(ethenyloxy)- Octadecane	0.34
10	10.21	1-Nonanol	1.84
11	10.39	7-Tetradecene	0.73
12	11.01	Terpinen-4-ol	2.28
13	11.14	Naphthalene	1.18
14	11.98	α-pentyl- Benzenemethanol	0.77
15	12.42	4-propyl- Benzaldehyde	0.74
16	14.04	Tetradecane	3.29
17	14.75	3,4-Dimethoxyphenethyl alcohol	2.57
18	15.29	Unidentified	2.04
19	15.80	3-ethyl-5-(2-ethylbutyl)- Octadecane	2.49
20	16.51	Heptacosane	6.30
21	17.05	1,5,5,8α-tetramethyl-, [1R(1à,3aá,4à,8aá,9S*)]- decahydro- 1,4-Methanoazulen-9-ol	0.67
22	17.21	1,5,5,8α-tetramethyl-, [1R(1à,3aá,4à,8aá,9S*)]- decahydro- 1,4-Methanoazulen-9-ol	0.49
23	17.65	17-Pentatriacontene	0.37
24	18.25	Heptacosane	1.73
25	18.65	Behenic alcohol	18.92
26	19.56	17-Pentatriacontene	2.55
27	20.07	2,5-di-tert-Butyl-1,4benzoquinone	6.06
28	20.45	1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester	2.58
29	20.73	Unidentified	5.04

30	21.33	(Z)- 9-Octadecen-1-ol	2.89
31	21.51	eicosyl ester of Oleic acid	2.42
32	21.70	eicosyl ester of Oleic acid	2.55
33	22.43	3-ethyl-5-(2-ethylbutyl)- Octadecane	0.62
34	22.57	Unidentified	2.63
35	23.43	Unidentified	1.66
36	24.26	Unidentified	2.72
37	25.07	Unidentified	2.42
38	25.52	Bis(2-ethylhexyl)phthalate	4.47
39	28.03	Octamethyl- Cyclotetrasiloxane	2.63

4. Discussion

Medicinal plants since time immemorial have been used in virtually all cultures of medicines. Traditional medicinal system makes use of a wide range of natural products derived from plant, animal as well as mineral origin[18]. Increased interest towards natural products since last decade led to few plants under scarcity, whereas few are adulterated with spurious material [19]. Hence quality issues of these natural products are of major importance. Anatomical features, chemical constituents, activity profile are three major quality assurance area related to raw material standardization. Therapeutic activity of an herbal material is because of its secondary metabolites like alkaloids, tannin etc. Identification, quantification, separation of chemical constituent is foremost steps in chemical standardization of herbal drug [20].

Modern techniques like TLC, HPTLC, HPLC, GC have made chemical standardization technique a much more reliable and reproducible method for the standardization of either single herb or formulations. Chromatographic fingerprint profile represents qualitative/quantitative determination of various components present in a complex mixture of herbal product, or a single herb with chemical complexity. HPTLC (High performance thin layer chromatography) is a sensitive, fast, reproducible best method for separation and quantification of plant extract involving multiple chemical constituents[21].

Gas chromatography (GC) is a separation technique capable of separating highly complex mixtures based primarily upon differences of boiling point/vapour pressure and of polarity. It is more beneficial in separation and identification of volatile sample [22].

Pandanus odoratissimus L. the fragrant inflorescence containing a volatile oil mixture used in Indian system of medicine as a therapeutic agent. Various formulations are also prepared out of this single drug. Chemo-profiling of this drug through chemical standardization techniques like HPTLC and GC to separate, identify, thereby generate quality standards have been tried.

Male Inflorescence of *Pandanus odoratissimus* L. Collected, shade dried, cut in to small pieces. For HPTLC n-hexane extract of this sample was applied on a precoated aluminium plates using Benzene as mobile phase. Developed plates were visualized in UV 254, 366, under white light and then derivatised with vanillin sulphuric acid and scanned under UV 254 and 366 nm. HPTLC photo documentation has shown 5 peaks at 254nm, whereas 6 peaks at 366nm. After post derivatisation at 620nm drug has shown 9 peaks.

Volatile oil in the test drug was extracted using Clevenger's apparatus, methylated and sent for the Gas Chromatography. The results were tabulated as per the retention time.It has shown presence of totally 39 compounds among which 31were identified by their Mass spectra with MS-Library. 2,4-dimethyl-Pentane, Tetrachloroethylene, 3-Hexanone, Tetradecane, α-pentyl- Benzenemethanol are few major compounds separated and identified.

5. Conclusion

Chemical constituents of a raw material used in therapeutics are responsible for its effectives in treatment. Chemical standardization techniques like HPTLC, GC help in separation, quantification and identification of chemical constituents of herbal material; thereby generated chemo-profile can be used for further study.

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