Analysis of secondary metabolites in *T. argentea* pollen by GC-MS

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Abstract
Ornamental plants produce large quantity of flowers and hence pollen. Pollen contain number of metabolites require for pollen function which plays specific role in pollen pigmentation and UV protection during pollination. In the present investigation flavonoids and other secondary metabolites were analysed in *T. argentea* pollen. Extraction was carried out using ethanol: water (3:1), and were analysed by Gas Chromatography- Mass Spectroscopy (GC-MS). Different peaks represent the different secondary metabolites which were identified using NIST library search. *T. argentea* pollen is source of flavonoids and can be utilise for drug developments.

Keywords: Secondary metabolites, pollen, GC-MS

1. Introduction
Pollen is most crucial structure in plant life as it transfers male gamete and also carries of genetic material for successful fertilization. Reproductive capacity of pollen ultimately determines the seed production. This event is reached by successful pollination. During the course of pollination, pollen is independent of parent plants and mechanically travels by agent term as pollinator (Pacini, 1992) [1].
Colour of flower and pollen mainly pay attention of pollinators. Yellow colour of pollen is due to presence of caretenoid and flavonoids. Flower and pollen colour absorbs the light at shorter wavelength; which is visible to insect in ultra violet range of spectrum (Taiz and Zeiger, 2002) [2].
Amongst the secondary metabolites presence of flavonoides not only showed by antemophilous (pollination by insect) pollen. It indicates the defensive role of flavonoides in pollen. As pollen pigment content flavonoid that absorbs the light with shorter wavelength (UV light); which protect the genetic material (pollen contents) from UV radiation in the environment. It is reported that air born pollen contain higher amount of UV screening pigments than insect transmitted pollen (Linskens, 1992 and Bhattacharya *et al.*, 2006) [3-4].
Tabebuia argentea (Bur. and Schum.) is tree, belonging to the family Bignoniaceae. Flowering season from March to May and commonly grow as avenue plant. Present investigation is undertaken to know the secondary metabolites in the pollen of *Tabebuia argentea* (Bur. and Schum.) which may be utilized in pharmaceuticals preparations.

2. Material and Methods
2.1 Pollen collection
Pollen grains of *Tabebuia argentea* (Bur. and Schum.) were collected before anther dehiscence. Anthers were allowed to dehisce in the oven and sieved through meshes. The fresh pollen grains were allowed to dry in oven at 60 ^{0}C for three days. Dried pollen were homogenized and used for extraction.

2.2 Preparation of extract
Extraction was carried out in ethanol: water in 7:3 rations for 24 hours by mechanical vibration at room temperature. Extracts were evaporated to dryness. This dried extract was diluted with methanol and was proceed for identification of secondary metabolites.

2.3 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis
The GC-MS analysis of extract isolated from *Tabebuia argentea* (Bur. and Schum.) was carried out by employing 2 µl of sample. The GC-MS analysis was carried out using Alegent Hp 7880 with column of 30 meter length, 0.25 mm ID, 0.32 thickness. Helium gas is used as carrier gas at constant flow rate of 1ml/ minute. Injector temperature was set at 1000 C. The oven temperature were programmed from 500 C to 2800 C at 100 C/ minute to 2000 C then100 C/3 minutes to 2500 C ending with a 5 minutes isothermal at 2800 C. The sample was injected in split mode as 50:1.

2.4 Identification of compounds
Identification of the compounds was carried out by comparing the spectral data of sample with reference spectra in spectral libraries (NIST).

3. Result and Discussion
The GC-MS analysis of pollen shows the presence of important bioactive compounds. by using the gas chromatogram which gives the many peaks and relative concentrations of various compounds were calculated. The height of the peak corresponds to the relative concentration of compound. The GC-MS analysis produce fragmentation pattern of different bioactive compound. This fragmentation pattern is compared to the compounds present in reference library (NIST) which help to determine the structure of compounds. The GC-MS analysis provides chemical fingerprint of *T. argentea.*
The result revealed the presence of major identified compounds are 9, 12, 15-Octadecatrienal (42.60%), n-Hexadecanoic (32.54%) and (25S)-3Beta-acetoxy-5alpha, 22beta-spirost-9(11)-en-12beta-ol (5.325%) in the pollen of *T. argentea* (Table 1 Fig. 1).

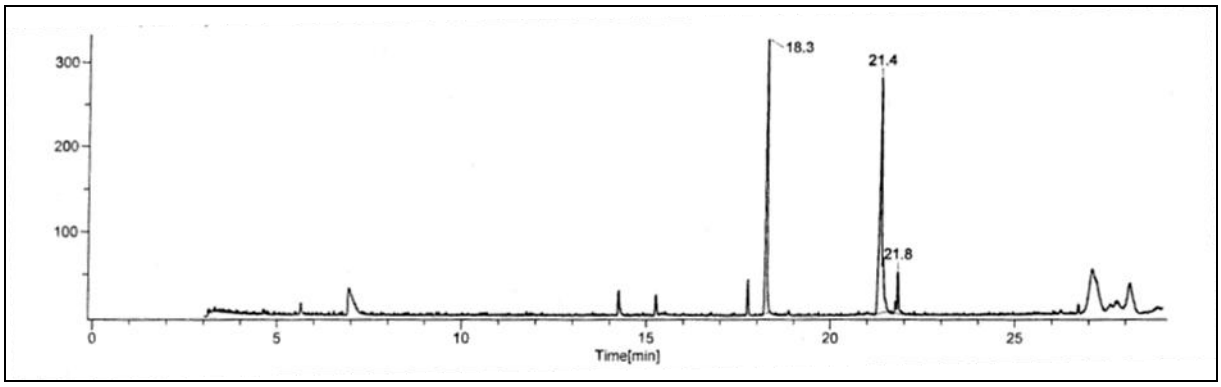

Fig 1: Chromatogram of extract of *T. argentea* pollen

Table 1: Compounds identified in pollen

Sr. No.	RT	Name of Compound	Peak area %	MW	MF
1	18.3	n-Hexadecanoic acid	32.54	256	$C_{16}H_{32}O_2$
2	21.4	9,12,15-Octadecatrienal	42.60	262	$C_{18}H_{30}O$
3	21.8	(25S)-3Beta-acetoxy-5alpha,22beta-spirost-9(11)-en-12beta-ol	5.325	472	$C_{29}H_{44}O_5$

RT= Retention Time, MW= Molecular Weight, MF= Molecular Formula.

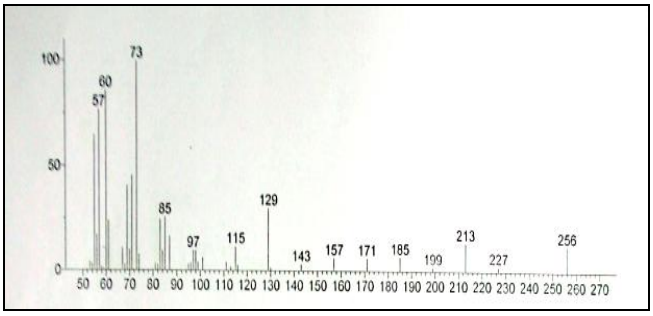

Fig 2: Mass spectrum of n-Hexadecanoic acid

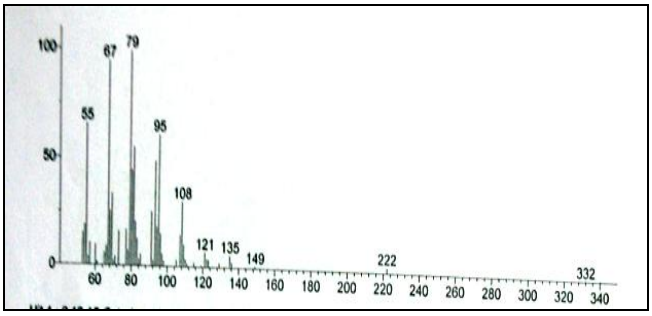

Fig 3: Mass spectrum of 9, 12, 15-Octadecatrienal

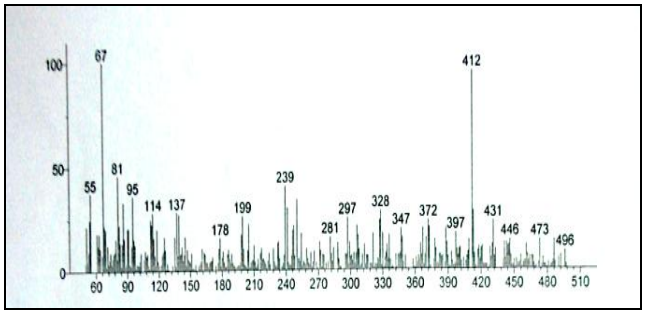

Fig 4: Mass spectrum of (25S)-3Beta-acetoxy-5alpha, 22beta-spirost-9(11)-en-12beta-ol

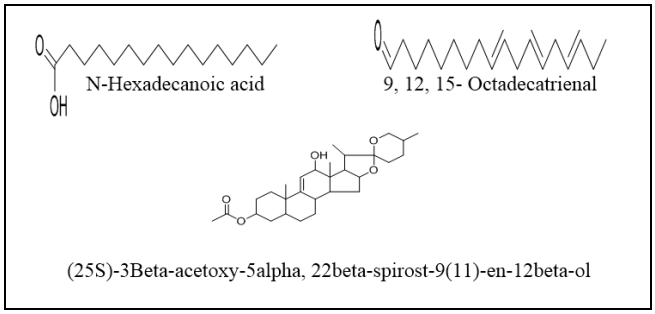

Fig 5: Structure of secondary metabolites in pollen of *T. argentea*

The present result revealed the presence of n-Hexadecanoic, 9,12,15-Octadecatrienal and (25S)-3Beta-acetoxy-5alpha,22beta-spirost-9(11)-en-12beta-ol in the pollen of *T. argentea.* n-Hexadecanoic acid is one of the constituent in pollen (Fig. 2) commonly known as Palmitic acid. Palmitic acid is mainly used to produce soaps, cosmetics, and release agents. Hydrogenation of palmitic acid yields acetyl alcohol, which is used to produce detergents and cosmetics. 9,12,15-Octadecatrienal also known as Octadeca-9,12,15-trienal. Its stereoisomer is Linolenyl aldehyde, Linolene aldehyde. (25S)-3Beta-acetoxy-5alpha,22beta-spirost-9(11)-en-12beta-ol has been reported in the pollen (Fig.4) which is also known as AGN-PC-O3M9XU, 25S)-3Beta-acetoxy-5alpha, 22beta-spirost-9(11)-en-12beta-ol. The presence of flavonoid has been reported in pollen (Markham and Camposa 1996) which may help in pigment formation in pollen (Ferreresa *et al.*, 1989) [5]. Chemically pollen rich in amino acids, fatty acids, flavonoid, phenolic acids (Stanley and Linskens, 1974) [6]. Due to teh prersence of secondary metabolites pollen shows antioxident properties (Fatrcová-Šramková *et al.*, 2013) [7].

4. Conclusions

The pollen acts as reliable source of secondary metabolites. n-Hexadecanoic acid is one of the constituent in pollen and has important commercial and cosmetics properties and can be

used in developing cosmetics and detergents. The rest of chemical constituent properties is still unknown and needed further research. Secondary metabolites may utilise for drug developments, medicines, cosmetics and pharmaceuticals.

5. Acknowledgement
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6. References

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