Profiling of Chemical Constituents in *Argyreia nervosa* Fruits using Modern Techniques

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Abstract:

In the present investigation the fruits of *Argyreia nervosa* Burm. were analysed by using methanol and dichloromethane solvent. Both samples were tested for preliminary qualitative characteristics for their bioactive compounds followed by gas chromatography – mass spectroscopy analysis. The preliminary qualitative tests of fruits indicated the presence of steroids, alkaloids, phenols and terpenoids in extracts. The GC – MS analysis revealed the presence of important bioactive compounds like Lysergamide, Stigmastan-3,5-diene, Ergotamine etc. whish are very useful for plants in ecological point of view and can be used in various medicine and drug formulations.

Keywords: *Argyreia nervosa*, GC – MS, Phytochemical Analysis, Bioactive compounds.

Introduction:

Argyreia nervosa belongs to family Convolvulaceae, commonly known as Elephant creeper. According to Biswas *et al.*, (1847) and Batra and Mehta, (1985), the seeds yielded fatty oils that are found to contain the glycerides of palminate, stearic, linoleic, linolenic and oleic acids (Biswas *et al.*, 1847;Batra and Mehta, 1985). In another study led by Kelkar *et al.*, (1947), the seed oil revealed the presence of myristoleic, myrisic, palmitic, linaloic, linolenic, oleic, stearic, nonadecanoic, eicosenoic, heneicosanoic and behenic acids identified in their corresponding methyl esters through GLC (Kelkar, *et al.*, 1947). The ethanolic extract of the seeds revealed the presence of a mixture of three alkaloids, out of which only one was characterised as ergometrin. Agarwal and Rastogi, (1974) extracted other constituents viz. caffeic acid and ethyl caffeate (Agarwal and Rastogi, 1974). In one another study also revealed the presence ergoline alkaloids in the seeds (Nair, *et al.*, 1987). They state that the ergolines were indicated to be of clavine type. The free amino acids reported in the seeds were glutamic acid, glycine, isoluecin, leucin, lysine, phenylalanine, tyrosine, proline and alpha-amino butyric acid (Jaiswal, *et al.*, 1984). The fruits were reported to contain n-tricontanol, beta-sitosterol, p-hydroxycinnamoyloctadecanolate and caffeic acid (Purushothaman *et al.*, 1982). This forgiving account of literature suggests that many phytoconstituents were reported from the *Argyreia nervosa* fruit mostly on the basis of GLC retention times. In the present investigation the fruit extracted in methanol and dichloromethane were subjected to GC as well as MS studies which will produce more faithful results.

Material and Methods:

A. Collection of plant material: The fruits were collected from the plant growing in University Campus, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India. The collected fruits were carefully examined for too old, infected parts and were removed accordingly. Only fresh fruits were selected for the analysis. The fruits after washing with tap water, dried in the shade till all its

moisture get evaporated. These dried fruits then converted to the powder form for further analysis.

B. Extraction: 10 gram of powder was extracted using Soxhlet apparatus for 24 hours in 180 ml of methanol and dichloromethane solvent. This extract was used for preliminary qualitative tests.

Preliminary Tests: Preliminary qualitative analysis was performed by using standard tests proposed by Herborne and Raaman.

Gas Chromatography – Mass Spectroscopy Analysis (GC – MS): 10 gram of powder was extracted using Soxhlet apparatus for 24 hours in 180 ml of methanol and dichloromethane solvent. This extract is then taken to dryness. This dried extract was dissolved in the 5 ml of methanol and dichloromethane solvent. 2 µl of this solution is employed for GC – MS analysis. 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 100^{0}C. The oven temperature were programmed from 50^{0}C to 280^0 C at 10^0 C/ minute to 200^{0}C then10^{0}C/3 minutes to 250^{0}C ending with a 5 minutes isothermal at 280^{0}C. The sample was injected in split mode as 50:1.

Identification of Compounds: Identification of compound was done by using various databases. The spectra of unknown compound ware compared with the spectra of known compound in the library. Mass spectral databases used for the comparing of mass spectrums are National Institute of Standard and Technology (NIST), Respect for Phytochemical Database, Metlin Mass Spectrums and Mass Bank Spectrums.

Observations: The fruit powder extracted in methanol and dichloromethane solvent was firstly analysed for the presence of important secondary groups. The observations of preliminary phytochemical analysis were showed in table no. 1. Both the extracts then analysed by gas chromatography and mass spectroscopy resulted in the identification of 12 and 4 compounds respectively in methanol and dichloromethane extracts. Both these results were placed in table 2 and table 3 respectively.

Discussion:

Fruit extracted in two solvents demonstrate the presence of alkaloid and phenols in both the extracts. Steroids and terpenoids were only found in dichloromethane extracts. Tannins and glycosides found only in methanol extracts.

Fruit extracted in methanol evinced the presence of 4H-Pyran-4-one, 2,3-dihydro-3,5-hydroxy-6-methyl, Lysergamide, 2-furancarboxaldehyde 5-(hydroxymethyl)-, 2,1,3-Benzothiadiazole and 2- Benzothiazolinethione 3-(O-chloroanilino methyl) as secondary metabolites while DL-Arabinitol, Palmitic acid and 9,12-Octadecadienoic acid are as primary metabolites.

4H-Pyran-4-one, 2,3-dihydro-3,,5-dihydroxy-6-methyl mass spectrum shows the mass peak and base peak at 144. Other fragments includes the 126, 115, 101, 97,

82, 68, 54, 43 etc. Which are accurately matching with standard NIST mass spectra of 4H-Pyran-4-one, 2,3-dihydro-3,,5-dihydroxy-6-methyl. **Lysergamide** spectrum shows the base peak at 72 and other fragments at 127, 109, 103, 91, 85, 69, 61, 55 etc. **2-furancarboxaldehyde, 5-(hydroxymethyl)-** mass spectrum shows the base peak at m/z 97 and mass peak at 126 and other major fragments at m/z 123, 109, 95, 81, 69, 55 and 41. Also known as hydroxymethylfurfural is a organic compound found as result of dehydration of certain sugars. This compound is yellow in colour and highly soluble in water. Its structure contains the furan ring, aldehyde and alcohol functional groups. It is observed in variety of heat processed foods such as milk, fruit juice, spirits, honey etc. Hydroxymethylfurfural is obtained from cellulose without the use of fermentation and is a potential source of carbon neutral feedstock for fuels and chemicals. Its derivative 5-hydroxy methyl-2-furfural has been found to bind specifically with intracellular sickled haemoglobin and inhibits the formation of sickle cells in blood. Higher quantities of hydroxymethylfurfural can be found naturally in coffee and dried fruits (NIST Webbook; Putten *et al.*, 2013, Huber *et al.*, 2006; Abdulmalik *et al.*, 2005; Murkovic and Pichler, 2006). 2,1,3-**Benzothiadiazole** spectrum shows the base peak at 136 and mass peak at 140 with other fragments such as 118, 110, 89, 77, 68, 63, 55, 51 etc**. 2- Benzothiazolinethione 3-(O-chloroanilino methyl)** mass spectrum shows the base peak at 73 and mass spectrum at 306 with other fragments such as 284, 264, 241, 227, 213, 199, 185, 171, 149, 135, 129, 121, 108, 95, 79, 67, 60, 55, 41 etc. Palmatic acid found in methanol extract is reconfirmed compound as its previously reported from the fruits of *Argyreia nervosa*. 9,12-Octadecadienoic acid also known as linoleic acid is the natural plant fatty acid.

Fruit extracted in dichloromethane shows the presence of Stigmastan-3,5-diene, Ergotamine, Azulene and 2-hydroxybenzoic acid, 2,4-dichlorophenyl ester. **Stigmastan-3,5-diene** mass spectrum shows the base peak at 206 and mass peak at 396 with other fragments at 288, 281, 280, 206, 147, 154, 81, 55 etc. **2-hydroxybenzoic acid, 2,4-dichlorophenyl ester** demonstrate the mass peak at 281 and base peak at 206 with other fragments at 100, 85, 57 etc. **Ergotamine** mass spectrum shows the base peak at 206 and mass peak at 581 with other fragments at 510, 451, 441, 351, 281, 280, 286, 192, 91, 85, 69, 55 etc. Ergotamine is used as medication since the middle ages, when the mass poisoning by ergotamine occurred throughout the Europe due to the eating of bread contaminated with the sclerotia of the mash room *Claviceps purpurea* which is known to be the parasite of rye, wheat, barley and other cultivated grains. These ergot alkaloids are obtained from naturally occurring ergot, which is the family of interrelated molecular entities that shares the ergoline ring system. It was first isolated from ergot fungus in 1918 and introduced as migraine therapy in 1926 as a sympatholytic drug. Due to its remarkable uterotonic and vasoconstrictor effects ergotamine is used in child birth and control of post-mortem haemorrhage (Thoms, 1931; Tfelt and Saxena, 2000; Silberstein, 1997; Schweinitzer and Weidmann, 1978). Beside this, ergotamine also used as hallucinogenic, haemostat, oxytocic and pesticidal (Dukes Database).

Table 1: Preliminary Phytochemical Analysis of Fruits

Sr.No.	Name of test	Dichloromethane	Methanol
1.	**Alkaloids**		
a.	Mayers reagent	+	+
b.	Dragendroffs reagent	+	+
c.	Wagner Reagent	+	+
2.	**Phenols**		
a.	Ferric chloride test	+	+
b.	Gelatin test	+	+
c.	Lead acetate test	+	+
3.	**Steroids**		
a.	Libbermann-Burchard test	+	-
b.	Salkowaski test	+	-
4.	**Tannins**		
a.	Gelatin test	-	+
b.	Lead acetate test	-	+
5.	**Saponins**		
a.	Froth test	-	-
b.	Foam test	-	-
6.	**Flavonoids**		
a.	Alkaline Reagent Test	-	-
b.	Lead acetate test	-	-
c.	Zinc powder test	-	-
7.	**Glycosides**		
a.	Borntragers test	-	+
b.	Killer-killani test	-	+
8.	**Terpenoids**		
a.	Copper acetate test	+	-
b.	Salkowaski test	+	-

Table 2: Phytoconstituents identified in Methanol Extract of Fruit.

S.N.	R.T.	Name of Compound	R. %	MF	MW
1	5.0	4H-Pyran-4-one, 2,3-dihydro-3,5-hydroxy-6-methyl	1.02	$C_6H_8O_4$	144.04
2	5.3	Lysergamide	6.42	$C_{16}H_{17}N_3O$	267.32
3	5.9	2-furancarboxaldehyde, 5-(hydroxymethyl)-	2.95	$C_6H_6O_3$	126.11
4	9.7	2,1,3-Benzothiadiazole	7.56	$C_6H_4N_2S$	140.21
5	15.9	DL-Arabinitol	2.25	$C_5H_{12}O_5$	152.15
6	17.4	Palmitic acid	17.35	$C_{16}H_{32}O_2$	256.42
7	20.3	9,12-Octadecadienoic acid	28.27	$C_{18}H_{32}O_2$	280.45
8	20.8	2- Benzothiazolinethione, 3-(O-chloroanilino methyl)	4.65	$C_{14}H_{11}ClN_2S_2$	306.83

Table 3: Phytoconstituents identified in Dichloromethane Extract of Fruit.

S.N.	R.T.	Name of Compound	R. %	MF	MW
1	3.1	Azulene	6.35	$C_{10}H_8$	128.06
2	18.4	Stigmastan-3,5-diene	5.23	$C_{29}H_{48}$	396.69
3	25.6	Ergotamine	7.28	$C_{33}H_{35}N_5O_5$	581.66
4	26.5	2-hydroxybenzoic acid, 2,4-dichlorophenyl ester	2.26	$C_{13}H_8Cl_2O_3$	281.10

References:

Biswas B., Tiwari L.D. and Dutt S. (1947). Chemical composition of the fixed oil from the seeds of *Argyreia speciosa*. *Ind. Soap. J.* **13**: 51-54.

Batra A. and Mehta B.K. (1985). Chomatographic analysis and antibacterial activity of the seed oil of *Argyreia speciosa*. *Fitoterapia.* **56**: 357-359.

Kelkar G.M., Phalnikar N.L. and Bhinde B.V. (1947). Fatty oil from the seeds of *Argyreia speciosa* Sweet. *J. Indian Chem. Soc.* **24**: 83-86.

Agarwal S.K. and Rastogi R.P. (1974). Ergometrine and other constituents of *Argyreia speciosa* sweet. *Ind. J. Pharmacol.* **36**: 118-119.

Nair G.G., Daniel M. and Sabnis S.D. (1987). Ergolines in the seeds of some Indian convolvulaceae. *Indian J. Pharm. Sci.* **49**: 100-102.

Jaiswal S., Batra A., Verma S. and Bokadia M.M. (1984). Free amino acids of some regionally available medicinally important plant seeds. *Sci. Cult.* **50**: 24-26.

Purushothaman K.K., Arvada A. and Loganathan D. (1982). Phytochemical study of *Argyreia speciosa* (Vridhadaru). *Buli Med. Ethnobot. Res.* **3**: 250-253.

National Institute of Standards and Technology. NIST Chemistry WebBook. http://webbook.nist.gov/chemistry.

Putten R., Waal J.C., Jong E., Rasrendra C.B., Heeres H.J. and Vries J.G. (2013). Hydroxymethylfurfural, A Versatile Platform Chemical Made from Renewable Resources. *Chem. Rev.* **113**: 1499 – 1597.

Huber G.W., Iborra S. and Corma A. (2006). Synthesis of Transportation Fuels from Biomass: Chemistry, Catalysts, and Engineering. *Chem. Rev.* **106**: 4044–4098.

Abdulmalik O., Safo M.K., Chen Q., Yang J., Brugnara C., Ohene-Frempong K., Abraham D.J. and Asakura T. (2005). *Br. J. Haematol.* **128**: 552–561.

Murkovic M. and Pichler N. (2006). *Mol. Nutr. Food Res.* 50: 842–846.

Thoms H. (1931). John Stearns 1808 Account of the *pulvis parturiens*, a remedy of quickening childbirth. *Am J Obstet Gynecol.* **2**: 418-423.

Tfelt-Hansen P., Saxena P.R., Dahlof C., Pascual J., Lainez M., Henry P., Diener H.C., Schoenen J., Ferrari M.D. and Goadsby P.J. (2000). Ergotamine in the acute treatment of migraine A review and European consensus. *Brain.* **123**: 9–18.

Silberstein S.D. (1997). The pharmacology of ergotamine and dihy-droergotamine. *Headache*. **37**: 15–25.

Schweinitzer M.E. and Weidmann H. (1978). *Basic pharmacological properties: Ergot alkaloids and related compounds*. Handbook of experimental pharmacology. Berlin: Springer-Verlag. 49: 87–232.

Dr. Duke's Phytochemical and Ethnobotanical Databases (2009). Green Pharmacy Garden, Fulton. Available on-line at: http://www.ars-grin.gov/cgi-bin/duke/ethno-bot.pl ethnobot.taxon=Rumex%20 obtusifolius.