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In vivo and *in vitro* primary metabolite profiling of 15 selected medicinal plants

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Moringa oleifera,
Phyllanthus niruri,
Sapindus emarginatus,
Terminalia belerica,
Trigonella foenum graecum,
Withania somniferum.

ABSTRACT

Biochemical studies of the individual plant parts is a necessary prerequisite in order to evaluate their importance in the over all metabolism of the plant. In the present study various plant parts of selected 15 medicinal plants were evaluated, separately for their metabolite content. Maximum amount of total soluble sugars and lipids were present in *S.emarginatus*, starch in *D.indica*, protein in *P.niruri* and phenols in *A.squamosa*. The seeds of all the experimental plants showed more content of metabolites as compared to the other plant parts.

Introduction

Biochemical studies of the individual plant parts is a necessary prerequisite in order to evaluate their importance in the over all metabolism of the plant, as well as the role of specific substances that may be produced as direct or indirect products of metabolism in same physiological processes. Metabolism comprises coordinate series of coupled enzymatic conversions in living organisms. Hence, carbohydrates, proteins, amino acids, chlorophyll, vitamins, hormones, phenols etc are basic building blocks of plant without which the plant life is hampered.

Carbohydrates comprise the bulk of organic matter of plants having storage and skeletal function, in addition to their involvement in the basic metabolism of all organisms. Comparative distribution of carbohydrates in the plant kingdom has been well studied (Pigman, Bell, 1962). Different methods for determining reducing sugars, sucrose and starch have been described in detail (McCready, 1950; Loomis, 1973; Cronin & Smith, 1979; Niranjan & Katiyar, 1979).

Proteins are complex nitrogenous biopolymers and are ubiquitous components of all living tissues. They have indispensable function in cellular architecture, catalysis metabolic regulation and are an important weapon in the defense arsenal of many higher organisms. Plant proteins have been well reviewed (Utsumi & Makoto, 1987) and a method for estimation of total protein content had been described by different workers (Lowry *et al*, 1951; Osborne, 1962).

Lipids are molecular organic compounds, composed largely of carbon and hydrogen and are essential for cell growth. They combine with carbohydrates and proteins to form the major component of plant and animal cells.

Phenolic compounds comprise a wide range of plant metabolites, which bear at least one hydroxyl group attached to an aromatic ring system.

Various workers described that the phenolic substances are the active products of cellular metabolism and of great importance as they act as analog of growth hormones (Bray & Thorpe, 1954; Harborne, 1964, 1980; Walker, 1975). Based on their chemical structures more than 10 classes of polyphenols have been identified (Bravo, 1998.).

MATERIALS AND METHODS

Experimental Plants

Various plant parts of *Annona squamosa* L., (Annonaceae), *Cassia occidentalis* L., *C. tora* L., *Derris robusta* (Roxb.) Benth., *D.indica L., Medicago sativa* L., *Trigonella foenum-graecum* L., (Fabaceae) *Hygrophila auriculata (Schumach) Heine, H.quadrivulvis Buch-Ham*,(Acanthaceae), *Moringa oleifera* Lam., (Moringaceae), *Momordica dioica* Roxb.,(Cucurbitaceae), *Phyllantus niruri* L., (Euphorbiaceae), *Sapindus emarginatus* Vahl, (Sapindaceae), *Terminalia belerica* Roxb., (Combretaceae) & *Withania somniferum* L., (Solanaceae) were separated, dried and powdered for evaluation of various primary metabolites.

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Carbohydrates

Total Soluble Sugars

The dried and experimental plant materials (50 mg each) were homogenized in a mortar and pestle with 20 ml of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 min, the supernatants were collected separately and concentrated on a water bath (Loomis, 1973). Distilled water was added to make up the volume up to 50 ml and processed further for quantitative analysis.

Starch

The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5.0 ml of 52% perchloric acid (McCready, 1950). Later, 6.5 ml of water was added in each sample and the mixture was shaken vigorously for 5 min. One-ml aliquot of each sample was used for the estimation of carbohydrates using the phenol-sulfuric method (Dubois *et al* ,1951).

Proteins

The test samples (50 mg each) were separately homogenized in 10 ml of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4^{o}C for 24 h. These mixtures were centrifuged, separately and supernatants were discarded. Each of the residues was again suspended in 10 ml of 5% TCA and heated at 80^{o}C on a water bath for 30 min. The samples were cooled, centrifuged and the supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight at room temperature (Osborne, 1962). Each of the above samples (1ml) was taken and the total protein content was estimated using the spectrophotometer (Lowry *et al* , 1951).

Lipids

The test samples were dried, powdered and 100 mg was macerated with 10 ml distilled water, transferred to a conical flask containing 30 ml of chloroform and methanol (Jayaram, 1981). The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 ml of chloroform mixed with 2 ml of water were added and centrifuged. Two layers separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the pre weighed glass voiles and the colored aqueous layer of methanol which contained all the water soluble substances and thick pasty outer face layer were discarded in each test sample. The chloroform layers were evaporated to dryness and weighed. Each treatment was replicated thrice and their mean values calculated.

Total Phenols

The deproteinized test materials (200 mg each) were macerated with 10 ml of 80% ethanol for 2h, separately and left overnight at room temperature. The mixtures were centrifuged and the supernatants were collected separately and maintained up to 40 ml by adding 80% ethanol. Total Phenol content in each sample was thereby evaluated (Bray & Thorpe, 1951).

Results

The various plant parts of the experimental plants exhibited variation in total levels of various metabolites.

In *A. squamosa* amount of total soluble sugars were maximum in seeds (58.65 mg/gdw), which were at par with that of root, leaves and stem ranging from 30-34.23 mg/gdw. Out of two *Cassia* species analyzed, *C. tora* was found to be rich in total levels of various metabolites (total soluble sugar, 64.33mg/gdw; proteins, 442.5mg/gdw; lipids, 126.67mg/gdw; phenols, 27.78 mg/gdw**)** than that of *C.occidentalis* except starch, which was maximum in seeds of *C. occidentalis* (21.97 mg/gdw**).** Seeds of *C. tora* exhibited higher amount of all the metabolites except phenols, which was higher in leaves. Maximum content of starch was present in seeds and minimum in stem (19.56 and 11.89mg/gdw, respectively). Maximum amount of proteins was present in seeds (255 mg/gdw), lipids (80 mg/gdw) in leaves and phenols in root (70 mg/gdw).

Total soluble sugars and starch were in the range of 28.81-50.64 and 12.23-20.14 mg/gdw, respectively in *D. robusta*. Seeds exhibited the higher amount of protein content (240 mg/gdw) and lipid content (130 mg/gdw). Amount of phenols were almost at par in stem, seed and root in the range from 39.37-48.12mg/gdw. In *D. indica* maximum amount of total soluble sugars, proteins and phenols were in seeds (42, 580 and 34mg/gdw respectively) while lipids was found to be maximum in callus (22mg/gdw) that was at par with content in seeds (20mg/gdw) and minimum was in stem.

In *H. auriculata* maximum total soluble sugars and starch were observed in roots (60 and 127mg/gdw), while minimum in callus (18.2mg/gdw). Seeds had maximum protein content (670mg/gdw) and stem had minimum. Maximum lipids were found in seeds (17mg/gdw), while content in leaves and stem was at par (13mg/gdw). Phenols were maximum in seeds (7.5mg/gdw) and minimum in stem (2.4mg/gdw). In *H. quadrivalvis* maximum total soluble sugars were found in roots (55mg/gdw) that were at par with seeds (51mg/gdw) and minimum in callus (14.5mg/gdw). Maximum starch and proteins were observed in seeds (92mg/gdw and 710mg/gdw) and minimum in roots. Phenols were found to be maximum in seeds, which was at par with callus (6.5mg/gdw) and minimum in roots.

In *M. sativa*, the amount of total soluble sugar and starch were found to be at par in stem, leaves and seeds ranging from 76.17-77 and 21.39-22.39mg/gdw, respectively. Seeds showed the maximum amount of total proteins (356mg/gdw) and lipids (146.67mg/gdw), while total phenols content was maximum in leaves (6.22mg/gdw).

Maximum content of total soluble sugar, starch and proteins (80, 23.6 and 380 mg/gdw, respectively) were present in pods of *M. oleifera*. Maximum content of lipids and phenols were in stem (42 mg/gdw) and root (6.65 mg/gdw)**.** In *M. dioica*, fruits had the maximum content of total soluble sugars (55.16 mg/gdw), proteins (264 mg/gdw) and phenols (5.81 mg/gdw). The maximum content of starch (65.28 mg/gdw) and lipids (39.33) were present in roots.

In P. niruri maximum amount of total soluble sugars and starch were in leaves and minimum in roots. Roots had maximum amount of protein while stem had minimum. Callus had maximum content of lipids and roots had minimum. Phenols were found to be maximum in callus and minimum in roots.

Fruits of *S. emarginatus* had maximum amount of proteins (283.33mg/gdw) and minimum of phenols (4.49mg/gdw) while fruits of *T. belerica* also had same observation. Likewise, almost same amount of total soluble sugar (76.83-77.33mg/gdw) and starch (22.04-22.16mg/gdw) was found in aerial plant parts and seeds of *T. foenum-graecum*. Seeds exhibited the higher amount of total proteins (312.5mg/gdw) and lipids (110mg/gdw) content than the aerial plant parts whereas phenols content (6.18mg/gdw) was higher in aerial plant parts than seeds. In *W. somniferum* roots had maximum amount of total soluble sugars (57mg/gdw) and stem had minimum (20mg/gdw). Leaves had maximum amount of phenols (28mg/gdw) while protein and

lipid content was found to be maximum in fruits (295mg/gdw and 25.01mg/gdw) and minimum in stem.

Comparison

The 15 medicinally important plants selected for phytochemical investigation showed variation in total levels of various metabolites. Overall *A. squamosa, C.occidentalis, C.tora, D. robusta, T.belerica* were found to be good source of phenols.

Total soluble sugar content was maximum in fruits of *S. emarginatus* (82.33 mg/gdw), which was at par with pods and callus of *M. oleifera* (80 and 77mg/gdw, repectively) and all plant parts of *M. sativa*. However minimum content was found in roots of *D. indica* (17mg/gdw) that was found to be at par with callus of *D. robusta*. Starch was maximum in callus of *D. indica* (105mg/gdw) followed by fruits of *T.belerica* (99mg/gdw) and *S. emarginatus* (96.17mg/gdw). Minimum content was observed in callus of *D. robusta* and *A. squamosa.*

Protein content was maximum observed in roots of *P. niruri* (780mg/gdw) and minimum was in callus of *D. robusta* and roots of *D. indica*(93mg/gdw). Overall all plant parts of *P. niruri*, seeds of *D. indica,* seeds and callus of *H. quadrivalvis* were found to be good source of protein.

Lipids were maximum in fruits of *S. emarginatus* (283.33mg/gdw) followed by seeds of *M. sativa* (146.67mg/gdw) and *D. robusta* (130mg/gdw). Minimum content was observed in roots of *P. niruri* (4mg/gdw).

Phenols content were maximum in roots and stem of *A. squamosa* (70 and 61.25mg/gdw) and minimum was in roots of *H. quadrivalvis* (2.01mg/gdw).

Discussion

Niranjan and Katiyar (1979) evaluated the range of crude proteins (22-31%), total carbohydrates (9.68-11.80%) and total lipids (1.61-3.91%) in selected leguminous plants. Stem, leaves and seeds of *M.sativa* contained total soluble sugars and protein, which were found to be higher than those reported by (Duke, 1981a, 1982b). Aerial plant parts and seeds of *T. foenum-graecum (fenugreek)* also showed higher amount of total carbohydrates and protein compared to the amount reported earlier by Duke & Ayensu, (1985). Seeds of *C. tora* exhibited 44%higher content of protein than those reported by Oudhia (2002). In seeds of *C. occidentalis*, the level of sugar and starch (5.1 and 2.2 parts in 100g) were also found to be higher compared to the amount examined. All the metabolites, except proteins, were present in more quantity in *D. robusta* than in *D. indica*. Moreover, it was observed that in different plant parts of *A. squamosa*, proteins and lipids were in the range of 18.9-23% and 3-8% respectively, which was higher as compared to the content as reported in the fruits of *A.squamosa* by Pinto & Cordeiro (2005). Phenol content was highest in roots and stem of *A.squamosa* followed by *Cassia* sps and *D.robusta* compared to all other experimental plants plants.

The protein and lipid content of *M. oleifera* pods (2.5% and 0.1%, respectively) and leaves (6.7% and 1.7%, respectively) as compared to earlier findings (Fugile, 1994) was much less than the amount estimated in the present investigation. *M. dioica* has been reported to be rich in a carbohydrate (Rashid, 1976), which was also observed in the present investigation. There are other reports on the evaluation of primary metabolites in related species *M. charantia* (Lissil, 1980). The difference in the content may be attributed to the various geographical niches in which these plants must be growing (Butcher, 1977). *Hygrophila* sps have not been reported of metabolite content and therefore this is the first report.

In the present study, significantly higher levels of proteins in *P.niruri* (780mg/gdw) and lipids in *S.emarginatus* (283.33mg/gdw) and comparatively low content of carbohydrates (17mg/gdw) in *D.indica* was observed in evaluation of these metabolites in various plant parts. The present investigation also reveals that seeds of the selected experimental plants showed high content of carbohydrates (sugar and starch) and proteins and thus can be used as nutritional supplements singly or in combinations.

References

Pinto AC de Q., MCR Cordeiro, SRM de Andrade, FR Ferreira, HA de C. Filgueiras, RE Alves and DI Kinpara (2005) *Annona* species, International Centre for Underutilised Crops, Universty of Southampton, Southampton, UK

Bell DJ (1962) Carbohydrates. In: M. Florkin and H.S. Mason eds. Comparative Biochemistry. Academic Press, New York; **3**, pp. 288-355.

BPI (Bureau of Plant Industry). In: Medicinal Plant of the Philippines. Manila, Philippines (2005).

Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev.* **56**: 317-333

Bray HG and Thorpe WV **(**1954).Analysis of phenolic compounds of interest in metabolism. *Meth Biochem Anal.* **1**: 27-52

Butcher DN (1977). Secondary products in tissue cultures. In: J. Reinert and Y.P.S. Bajaj eds. Applied and fundamental aspects of plant cell, tissue and organ culture. Springer-verlag, Berlin; pp 668-693

Cronin DA and Smith S (1979). A simple and rapid procedure for the analysis of reducing total and individual sugars in potatoes. *Potato Res.* **22**: 99-105

Dubois M, Gills KA, Hamilton JK, Rebers PA and Smith F (1951). Colorimetric method for determination of sugars and related substances. *Anal Chem.* **28**: 350-356

Duke JA (1981 a) *Handbook of legumes of world economic importance*. (Plenum Press, NewYork,).

Duke JA (1981b) The gene revolution. Paper 1. p. 89-150. In: Office of Technology Assessment, Background papers for innovative biological technologies for lesser developed countries. USGPO. Washington.

Duke JA and Ayensu ES (1985). *Medicinal Plants of China* Reference Publications, Inc. ISBN 0-917256-20-4. Details of over 1,200 medicinal plants of China and brief details of their uses

Fuglie LJ (1999) *The Miracle Tree: Moringa oleifera: Natural Nutrition for the Tropics*. (Church World Service, Dakar, pp 68.

Harborne JB (1964). Phenolic glycosides and their natural distribution. In: J.B. Harborne ed. Biochemistry of Phenolic Compounds. Academic Press, London; pp.129-170).

Harborne JB, Plant phenolics (1980). In: E.A. Bell and B.V. Charlwood eds. Secondary plant products. **Vol. 8**. Springer-Verlag, Berlin-Heidelberg; pp.320-329

Jayaraman J. 1980. *Laboratory Manual in Biochemistry*, (Wiley Eastern Limited, New Delhi, 1981) pp. 96-97.

Lissil N. (1980). Purification and partial characterization of to lactones from *Momordica charantia* L. *Experimentia* **36(5)**: 524-527

Lowry O H, Rose HN, Broug J, Farr AL and Randall RJ (1951). Protein measurement with the Folin-phenol reagent. *J Biol Chem.* **193**: 265-275.

Loomis WE and Shull CA 1973. *Methods in Plant Physiology*, (McGraw Hill Book Co., New York,).

McCready RM, Guggoiz J, Silviera V and Owens HS (1950). Determination of starch and amylase in vegetables. *Anal Chem.* **22**: 1156-1158

Niranjan GS and Katiyar SK (1979).Chemical analysis of some wild leguminous seeds. *J Ind chem Soc.* **56(7)**: 722-725

Osborne DJ **(**1962).Effect of kinetin on protein and nucleic acid metabolism in *Xanthium* leaves during senescence. *Plant Physiol.* **37**: 595-602

Oudhia P. Charota or Chakod. (2002). New Crop Resource Online Program, Center for New Crops and Plant Procucts, Purdue University. USA

Pigman W, *The Carbohydrates*, (Academic press, New York).

Rashid MM (1976). Bangladesh Shabjee 1st ed. Bangla Academy, Dhaka, Bangladesh. P. 494

Utsumi S and Makoto K **(**1987). Seed proteins: Present situation and review of research and application. *Kagakuto Seibustu.* **25(1)**: 62-67 8.

Walker JRL (1975). Biology of Plant Phenolics. *Inst Biol Stud Biol.* **54**: 1-57

Yuwai KE, Rao KS, Kaluwine CK, Jones GD and Rivett DE (1991).Chemical composition of *Momorica charantia* Fruits. *J Agric Food and chem*. **39 (10)**: 1762-1763.

Table 1. Metabolite profile of various selected 15 medicinally important plants

Names of Experimental Plants	Plant Parts	Total Soluble Sugar	Starch	Proteins	Lipids	Phenols
	mg/gm dry weight					
Annona squamosa	Roots	30 .01± 1.11	15.78 ± 1.03	230 ± 1.30	30± 1.25	70 ± 0.86
	Stem	34.24 ± 1.01	11.89 ± 0.63	205 ±1.09	30 ±1.22	61.25 ± 0.14
	Leaves	32.45 ±0.63	12.15 ± 0.53	189 ±0. 43	80 ± 1.08	48.75± 0.74
	Seeds	58.65 ±1.26	19.56 ±0.75	255 ±0.93	35 ± 0.82	32.87 ± 0.66
	Callus	26.34 ± 1.02	9.08 ±0.62	150 ±1.19	20 ± 0.04	55.70 ± 1.23
Cassia occidentalis	Roots	44.50 ±0.58	17.83 ± 0.23	275 ±1.21	20 .00± 0.12	15.04 ±0.78
	Stem	38.17 ± 0.15	18.32 ± 1.14	290 ±0.64	26.67 ± 0.15	14.76 ± 0.46
	Leaves	41.00 ± 0.39	18.55 ±0.76	306 ± 1.24	70 .00± 0.26	22.13 ± 1.19
	Seeds	51.33 ± 0.71	21.97 ±0.66	434 ± 0.95	76.67 ± 0.22	27.53 ± 0.87
	Callus	25 .00± 1.12	12.00 ± 0.51	105 ± 0.49	30.00 ± 0.54	28.12 ± 0.44
C. tora	Roots	46.17 ± 0.08	17.71 ± 0.11	305.50 ± 1.11	30 .00±0.17	21.58± 0.34
	Stem	51.33± 0.54	19.36 ± 0.66	301 .00± 1.05	23.33 ±0.15	18.67 ± 0.15
	Leaves	37.17 ± 0.43	18.17± 0.37	404.50 ± 1.34	63.33 ±0.28	27.78 ± 0.66
	Seeds	64.33 ± 0.56	20.47 ± 0.43	442.5 0± 0.98	126.67± 0.95	17.62 ± 043
	Callus	22.00 ± 0.27	10.00 ± 0.22	120.00± 0.56	80.00 ±0.85	26.00 ± 0.27
Derris indica	Roots	17 ± 0.71	10 ± 0.81	93 ± 0.21	18 ± 0.12	11 ± 0.21
	Stem	20 ±1.41	15 ± 0.57	225 ±1.14	08 ± 0.01	10 ± 1.11
	Leaves	27 ± 1.31	28 ± 0.57	310 ±1.31	12 ± 0.13	25 ± 1.21
	Seeds	42 ± 1.42	35 ± 0.48	580 ± 1.04	20 ±1.05	34 ± 0.01
	Callus	29 ± 1.02	105 ±1.12	410 ± 0.01	22 ± 0.02	28 ± 1.01
D. robusta	Roots	28.81 ± 0.89	17.54 ± 0.58	115 ±0.53	70 ± 1.62	39.37 ± 1.09
	Stem	35.45 ±0.42	12.23 ± 1.02	90 ± 1.05	80 ± 0.68	48.12 ± 1.06
	Leaves	42.45± 0.43	14.11 ± 1.14	130 ± 0.05	110 ± 0.74	52 .5 ± 1.01
	Seeds	50.64 ± 0.42	20.14 ± 0.86	240 ± 0.89	130 ±1.20	45.62 ± 0.64
	Callus	18.07 ± 0.96	9.03 ± 1.03	85 ±1.19	50 ± 1.25	35.87 ± 1.24
Hygrophila auriculata	Roots	60.1± 0.36	127±0.42	350 ±0.76	12.01±0.22	2.4±0.02
	Stem	29.1±0.09	72.2 ±0.02	250 ±1.12	13 ±0.19	2.4±0.68
	Leaves	41.5±0.21	69.5±0.69	320 ±0.28	13±0.26	3.1±0.68
	Seeds	56.1±0.62	93.2 ±0.03	670 ±0.32	17±0.03	7.5± 0.66
	Callus	18.2±0.78	77.1 ±0.20	560 ±0.09	11±0.28	3.8± 0.11
H.quadrivalvis	Roots	55±0.01	59.01±0.19	200±0.12	13 ±0.23	2.01±0.22
	Stem	30±0.02	61.69±0.68	400±0.76	15 ±0.62	2.20±0.09
	Leaves	39±0.26	70.01±0.72	400±0.89	15 ±0.28	3.23±0.12
	Seeds	51±0.121	92.02±0.13	710±0.36	26 ±0.32	6.51±0.08
	Callus	14.5±0.22	87.11±0.12	570±0.11	16.±0.72	6.52 ±0.03
Medicago sativa	Stem	76.17± 0.38	22.39±0.11	247±0.99	76.67± 0.36	2.67±0.02
	Leaves	77.50± 0.33	21.39±0.12	304±1.13	60.00±0.22	6.22± 0.07
	Seeds	76.67±0.29	21.95±0.11	356±1.16	146.67±0.87	5.16±0.05
Momordica dioica	Roots	48.22±0.21	65.28±0.51	178±1.22	39.33±0.26	4.54±0.09
	Stem	39.67±0.38	34.53±0.49	198±0.87	31.45±0.19	4.67±0.10
	Leaves	46.11±1.01	41.45±0.78	214±0.49	30.12±0.22	5.13±0.13
	Fruits	55.16±0.56	51.41±0.56	264±1.12	28.24±0.11	5.81±0.12
	Callus	48.75±0.69	47.84±0.77	227±0.88	31.54±0.22	4.97±0.22
Moringa oleifera	Roots	45±0.69	19.70±0.73	140±1.19	24±0.89	6.65±0.33
	Stem	47±0.78	22.04±1.23	195±0.58	42±1.13	5.57±0.32
	Leaves	55±1.26	21.11±0.73	232.13±1.30	25±0.1	4.5±0.09
	Pods	80±1.03	23.6±0.94	380±1.75	33.00±1.25	3.2±0.13
	Flowers	70.±0.93	22.46±0.83	350.00±1.33	35.00±0.82	2.9±0.04
	Callus	77±.97	22.39±0.67	251.09±0.93	42.00±0.79	4.7±0.23
Phyllanthus niruri	Roots	35± 0.03	84±0.12	780±0.32	04±0.02	8.51 ±0.01
	Stem	61± 0.42	83±0.11	530±0.21	09±0.03	9.01 ±0.03
	Leaves	65± 0.38	86±0.21	540±0.43	13±0.11	14.02±0.03
	Callus	55± 0.41	80±0.31	580±0.22	15±0.13	20.03±0.05
Sapindus emarginatus	Fruits	82.33 ± 0.19	96.17± 0.37	205.33 ± 0.39	283.33 ± 2.73	4.49 ± 0.05
Terminalia belerica	Fruits	78.50 ± 0.78	99.00± 0.95	211.0 ± 0.41	46.67 ± 7.2	37.11 ± 0.08
Trigonella foenum- graecum	Aerial parts	76.83±0.35	22.16±0.11	265.50±0.99	73.33±0.08	6.18±0.03
	seeds	77.33±0.33	22.04±0.99	312.50±1.11	110.0±0.99	5.2-±0.02
Withania somniferum	Roots	57 ± 1.1	55± 0.1	180 ± 0.01	23.03 ± 1.2	20.81 ±1.2
	Stem	20 ± 0.1	22 ± 0.1	140 ± 1.01	12.01 ±1.0	23.81 ±0.3
	Leaves	26 ±0.3	25 ± 1.0	195 ± 1.2	14.02 ±0.01	28.01 ±0.1
	Fruit	55 ± 1.1	60 ± 1.2	295 ± 1.3	25.01 ± 0.1	23.02 ±1.0