Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 1, 2012

Research Article

IN VIVO AND *IN VITRO* BIOCHEMICAL ESTIMATION OF PRIMARY METABOLITES FROM *JATROPHA CURCAS*: AN IMPORTANT BIODIESEL PLANT

R.SETH*[1], R. SARIN[1]

[1]Laboratory of Bioactive and Natural Products, Department of Botany, University of Rajasthan, Jaipur, India.
*Email: thesisruchi@gmail.com

Received: 26 Aug 2011, Revised and Accepted: 19 Sep 2011

ABSTRACT

Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Since time immemorial, man has used various plant parts in the treatment and prevention of various ailments. They are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds. Plants provide biologically active metabolites and lead structures whose activities can be enhanced by manipulation through combinations with chemicals and synthetic chemistry. These metabolites can be exploited in the field of new drugs research and for the development of modified derivatives with enhanced activity and reduced toxicity. In present investigation, callus raised from leaf explant and different plant parts (leaves, stems and roots) of *Jatropha curcas* Linn. were evaluated for their biochemical estimation of primary metabolites quantitatively. Results showed that maximum callus was obtained on MS medium supplemented with a combination of IAA+ Kn. Maximum content of soluble sugars and proteins were found in callus and starch, lipid and phenolic contents were found in leaf. It further concludes that *Jatropha curcas* serves as a rich source of primary metabolites which can be used as raw material in industries.

Keywords: *Jatropha curcas*, Primary metabolites, Sugar; Starch, Protein, Lipid, Phenols

INTRODUCTION

Plants have served as an integral part of human civilization across the continents since time immemorial. They have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 B.C. and 800 B.C. give many references of medicinal plants. One of the remotest works in traditional herbal medicine is "Virikshayurveda", compiled even before the beginning of Christian era. Some of the traditional medicines are still included as part of the habitual treatment of various diseases[1,2]. Plants are like natural laboratories where a great number of chemicals are biosynthesized and provide the most important source of chemical compounds.

Primary metabolites are substances widely distributed in nature, occurring in one form or another in virtually all organisms. They are of prime importance and essentially required for growth and development of plants for example; sugars, proteins, lipids, and starch. In plants such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism[3]. Primary meabolites are mainly used as industrial raw materials, food or food additives. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites of pharmaceutical compounds such as antipsychotic drugs[4, 5, 6, 7]. Primary metabolites viz., chlorophyll, amino acids, nucleotides and carbohydrates have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. Many plants such as *Nerium indicum*, *Gloriosa superba*, *Ricinus communis* and *Euphorbia hirta*, *Alangium salviifolium*, *Withania Somnifera*, *Moringa oleifera*, *Pongamia pinnata*, *Commiphora wightii*, *Maytenus emarginata*, *Digera muricata* have been evaluated for their composition of primary metabolites[8, 9, 3, 10, 11, 12, 13, 14, 15, 16, 17].

Jatropha curcas commonly known as physic nut or ratanjyot is a large, coarse, erect, perennial, succulent, deciduous, monoecious, drought resistant, annual shrub or small tree (6m) with thick spreading glorious branches. It can attain a height of up to 8 or 10m under favourable conditions. The plant shows articulated growth, with a morphological discontinuity at each increment. The tree has a straight trunk and smooth, papery, thin often greenish or gray bark, masked by large white patches. Bark exudes copious amounts of milky latex when cut is made, which is soapy to touch but soon becomes brittle and brownish when it dries. The branches are ascending, glaborous and stout containing latex having dark green leaves. Flowers occur in racemes in a diachasial cyme pattern. Fruits are small, yellow, ellipsoid capsule-like in shape about 2.5-4cm long by 2-3cm in diameter. Normally 5 roots are formed from seedlings, one central and 4 peripheral. A tap root is not usually formed by vegetative propagated plants. It is adapted to arid and semi arid conditions. It is widely cultivated in tropics and subtropics as an ornamental plant and living fence.

Latex possesses anticancerous and antimicrobial properties[18, 19, 20]. Leaves are regarded as antiparasitic, applied to scabies; rubefacient for paralysis, treat rheumatic and muscular pains; also applied to hard tumors and used against malaria. Some of the ethnomedical uses of the extracts of *J. curcas* leaves and roots include use as a remedy for cancer, as an abortifacient, antiseptic, diuretic, purgative and haemostatic. The root methanol extract exhibited anti-diarrhoeal activity[21]. Seeds are used for dropsy, gout, paralysis, skin ailments and as a source of oil for stimulating hair growth. The oil from its seeds has been also found useful for medicinal and veterinary purposes[22]. The sap from twigs is considered styptic and is used for dressing wounds and ulcer. The tender twigs of the plant are used for cleaning teeth and juice of the leaf is used as an external application for piles. A decoction of bark is used externally for treating rheumatism and leprosy in Andhra Pradesh. The juice of the flowers has numerous medicinal uses. The present study was conducted to investigate biochemical estimation of primary metabolites viz., total soluble sugar, starch, phenol, proteins and lipids of *J. curcas*.

MATERIALS AND METHODS

Plant material

Healthy plants of *J. curcas* were collected from State Forest Research Institute, Jabalpur, Madhya Pradesh. Plant was authenticated by the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. Fresh leaves were taken from pot grown plants for callus culture. Callus and all the plant parts were dried and powdered with the help of pestle and mortar

Callus induction

Leaves were surface sterilized with 0.2% Cedepol for 10-15 minutes followed by washing with sterile distilled water to remove the detergent. After that, the explants were treated with 0.1% $HgCl_2$ aqueous solution for 4-5 minutes and then washed thoroughly with sterilized distilled water to remove traces of $HgCl_2$.

The leaves were then cut into pieces with sterile scalpel. Leaf discs were inoculated on to the MS medium[23] fortified with different concentrations of various auxin and cytokinin. The pH of the media was adjusted to 5.7±0.1 before autoclaving. All media flasks were sterilized by autoclaving at 121ºC and 15lbs/psi for 15-20 minutes. The cultures were incubated under carefully regulated temperature and light conditions in an air conditioned room under the ambient conditions (25 ± 2ºC; 55-60% RH) and 16 hour photoperiod. 20 replicate cultures were established and each experiment was repeated thrice and the cultures were observed at regular intervals.

Primary metabolite estimation

Callus, root, stem and leaf parts of *J. curcas* were evaluated quantitatively to estimate the total content of soluble sugars, starch, proteins, lipids and phenols following the established methods for the sugars, starch[24], protein[25], lipid[4] and phenol[5]. All experiments were repeated three times for precision and values were expressed in mean ± standard deviation.

RESULTS

Callus induction

MS medium supplemented with different concentrations of various auxins and cytokinins showed different response for callus induction. Leaf showed maximum callus formation on MS medium supplemented with a combination of IAA 5.0mg/L + Kn 2.0mg/L. The callus produced was green, irregularly lobed, compact and friable. It grew profusely and possesses high regenerative potential. The ageing of callus showed green color changing to brownish green (shown in Table1). Callus obtained after 8 weeks of culture from MS medium was further evaluated for primary metabolites.

Table 1: Percentage of callus induction from *J. curcas* leaves under different levels of IAA and Kn

S. No.	Phytohormones concentration (mg/l)		Callus induced explant (%)	Nature of callus
	IAA	Kinetin		
1	5.0	0.25	86 ± 5.16	greenish white friable
2	5.0	0.50	91 ± 3.16	greenish white friable
3	5.0	1.00	98 ± 4.21	greenish compact
4	5.0	1.50	100 ± 0.00	green compact
5	5.0	2.00	100 ± 0.00	green compact

Primary metabolites

Maximum amount of soluble sugars (73.5±0.18 mg/gdw) was found in callus in comparison to plant parts and minimum amount of soluble sugar (4.22±0.10mg/gdw) was found in stem. Highest amount of starch was found in leaves (6.52±0.06mg/gdw) while lowest amount was found to be present in stem (3.63±0.05mg/gdw). Callus was found to contain maximum amount of protein (41.25±0.17mg/gdw) and minimum amount was shown by root (23.16±0.04mg/gdw). Maximum amount of lipid (30.22±0.08mg/gdw) was exhibited by leaves while minimum lipids (19.73±0.09mg/gdw) were present in root. Leaves were found to contain highest amount of phenols (38.97±0.14mg/gdw) while minimum phenolic content was found in roots (18.17 ± 0.20mg/gdw). (Shown in table 2 and fig 1).

Table 2: Primary metabolites from *J. curcas in vivo* and *in vitro*

Plant Parts	Sugar mg/gdw	Starch mg/gdw	Protein mg/gdw	Lipid mg/gdw	Phenol mg/gdw
Leaves	9.24±0.07	6.52±0.06	40.37±0.17	30.22±0.08	38.97±0.14
Stem	4.22±0.10	3.63±0.05	30.22±0.11	27.47±0.04	22.30±0.09
Root	6.22±0.11	5.49±0.14	23.16±0.04	19.73±0.09	18.17±0.20
Callus	10.27±0.08	5.27±0.12	41.25±0.17	12.29±0.07	10.26±0.13

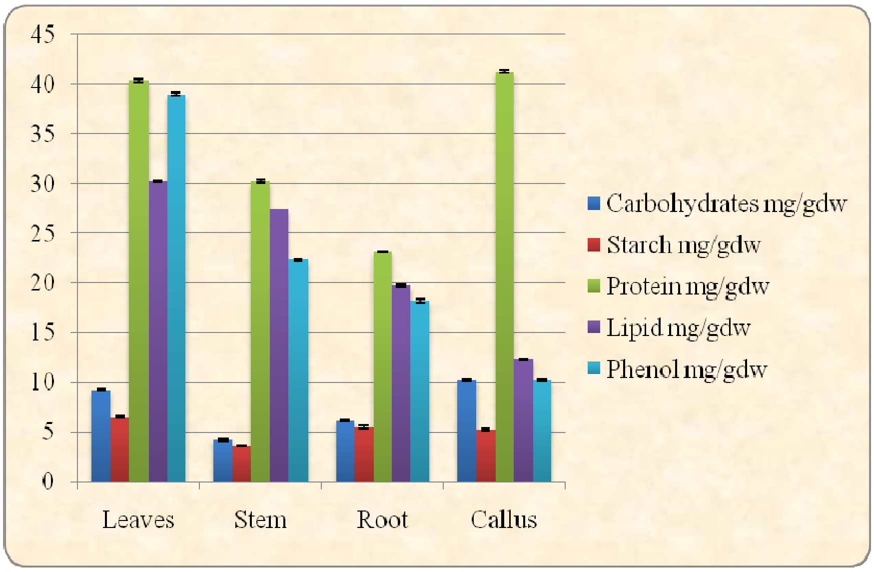

Fig. 1: Primary metabolites from various plant parts of *J. curcas*

DISCUSSION

For centuries plants are globally valued, as they hold medicinal, flavouring and aromatic qualities. Now days, there has been an increasing interest worldwide on therapeutic values of plants due to the presence of natural products.

Carbohydrates are important group of carbon compounds which are essential to sustain life. Numerous polysaccharides purified from Chinese medicinal herbs are bioactive and possesses immunomodulating, anti-tumour and antibacterial activities[26]. Sugar has large numbers of stereo-isomers, because they contain several asymmetric carbon atoms[27]. Previously, higher content of sugar was reported in callus of *W. somnifera* (73.5±0.18 mg/gdw) and *C. wightii* (78.0±0.42 mg/gdw)[11, 15]. Plant sugars can be used as artificial sweeteners and they can even help diabetics by supporting the body in its rebuilding[28]. *In vitro* cells accumulate more sugar due to its easy availability in culture medium and these cells are in highly proliferating stage so they accumulate more primary metabolites than storage metabolites (starch, lipid) and secondary metabolites (phenolic contents).

Similarly, higher starch content was reported in the leaves of *N. indicum* (6.00 mg/gdw), *W. somnifera* (44.2±1.32 mg/gdw), *M. oleifera* (42.0±0.60 mg/gdw) and *D. muricata* (33.5±1.41 mg/gdw)[8, 11, 12, 17]. Starch is one of the most abundant metabolite in plants. Wheat, potato and cassava mostly used as food are the major sources of starch[29]. It is biodegradable and renewable in nature. Starch is used in cosmetic formulation like face powder and in dusting preparations that use aerosol dispensing systems[30]. It may also be used as a substitute for petroleum based plastics[31]. Starch is being increasingly considered as an eco-friendly alternative to the use of synthetic additives in many other products, including plastics, detergents, pharmaceutical tablets, pesticides, cosmetics and even oil-drilling fluids[32] which show the potential of studied plants as the above mentioned sources.

Proteins are the primary components of living things. In similar studies carried out, amongst the plant parts protein content was maximum in leaves of *N. indicum* (40.00 mg/gdw), *W. somnifera* (40.8±1.15 mg/gdw), *D. muricata* (78.0±1.01 mg/gdw)[8, 11, 17]. The presence of higher protein level in the plant points towards their possible increased food value or that a protein base bioactive compound could also be isolated in future[33] with various activities.

Lipids are the supporters and storage molecules of cells. They provide a diverse group of primary metabolites including fats, essential oils, waxes, terpenoids and oleoresin. Previously, higher content of lipid was reported in leaves of *W. somnifera* (42.7±1.18 mg/gdw), *M. oleifera* (46.0±0.05 mg/gdw), *C. wightii* (30.0±0.45 mg/gdw) and *D. muricata* (18.3±0.71 mg/gdw) respectively[11, 12, 15, 17]. They are being used by industry as highly stable lubricant and as a renewable source of fuel[34, 35]. The higher amount of plant lipid can be used as essential oils, spice oleoresins and natural food colors. Increasing research and development has shown that lipids obtained from plants serves as products that work with diverse requirements, be it culinary, medicinal or cosmetic[34]. This could be the reason that the studied plant was used in folkloric medicines for various purposes.

Phenols play an important role in the precursor of toxic substances. In earlier studies phenols were found to be higher in leaves of *A. salviifolium* (53.8±1.60 mg/gdw) and *P. pinnata* (0.76 mg/gdw)[10, 14]. The higher amount of phenols is important in the regulation of plant growth, development and diseases resistance. They can be used as fungicide, pesticides, an antiseptic, disinfectant and in the manufacture of resins, explosives, plastics, detergents and pharmaceutical substances and have immunomodulating, anti-tumour and antibacterial activities[26]. Plant phenols may interfere with all stages of cancer process, potentially resulting in a reduction of cancer risk[36]. Higher amount of phenols was observed in the leaves of the plant, which could be one of the reasons of its antimicrobial, antioxidant and insecticidal potential.

CONCLUSION

Economic use of plants depends partially on the quantitative and qualitative aspects of there organic reserves, specially carbohydrates, proteins, phenols and lipids. In the present investigation highest amount of soluble sugar (10.27±0.08 mg/gdw) and protein (41.25±0.17 mg/gdw) was observed in callus, starch (6.52±0.06mg/gdw), lipid (30.22±0.08 mg/gdw) and phenol (38.97±0.14 mg/gdw) were observed in leaves as compared to other parts of the plant. This study suggests that plant parts having rich primary metabolites can be used industrially as raw materials having commercial importance. These primary metabolites could be further used for biosynthesis of secondary metabolites or bioactive compounds. Results obtained show the great interest in plant pharmaceuticals.

ACKNOWLEDGEMENT

The authors are indebted to the head, Department of Botany, University of Rajasthan for providing library and laboratory facilities. We also acknowledge State Forest Research Institute (SFRI), Jabalpur, Madhya Pradesh for providing the plant species. Similarly, we are thankful to the herbarium, Department of botany for assisting us with the identification of plant materials. Grateful acknowledgement is also due to the Professor C.K. Ojha Director (Academics), Mahatma Gandhi Institute of Applied Sciences, JECRC Foundation, Jaipur for their sincere suggestions and moral support during research work.

REFERENCES

1. Heinrich M, Barnes J, Gibbons S, Williamson EM: Fundamental of pharmacognosy and phytotherapy. Edinburgh, UK: Curchill Livingstone; 2004.
2. Rios JL, Recio MC: Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005; 100: 80-84.
3. Vijayvergia R, Sharma S, Singh T: Biochemical estimation of primary metabolites of some medicinal plants of Euphorbiaceae family. J. Indian. Bot. Soc. 2009; 88(1-2): 116-119.
4. Jayaraman J: Laboratory Manual in Biochemistry. New Delhi, India: Wiley Eastern Limited; 1981.
5. Bray HG, Thorpe WV: Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. 1954; 1: 27-52.
6. DeVane CL: Brief comparison of the pharmacokinetics and pharmacodynamics of the traditional and newer antipsychotic drugs. Am J Health Syst. Pharm. 1995; 52 Suppl 1: S15-S18.
7. Sanchez S, Demain AL: Metabolic regulation and overproduction of primary metabolites Microbial Biotechnol. 2008; 1(4):283-319.
8. Vijayvergia R, Kumar J: Quantification of Primary Metabolites of *Nerium indicum* Mill. Asian J. Exp. Sci. 2007; 21(1): 123-128.
9. Rishi A, Sarin R: Antimicrobial screening of *Gloriossa superba* L. and *Urginea indica* (Roxb.) KUNTH". Int.J. Mendel. 2009; 26: 29-30.
10. Tanwer BS, Vijayvergia R: Phytochemical Evaluation and Quantification of Primary Metabolites of *Alangium salviifolium*. Int J Pharma Bio Sciences. 2010; 1(3): 1-6.
11. Singh S, Tanwer BS, Khan M: Callus induction and in vivo and in vitro comparative study of primary metabolites of *Withania somnifera*. Adv. Appl. Sci. Res. 2011; 2(3): 47-52.
12. Sharma A, Yadav A, Barman N, Malwal M: Quantification of Primary Metabolites of *Moringa oleifera* Lam. The Bioscan. 2010; 5(3): 403-405.
13. Talreja T: Biochemical Estimation of Three Primary Metabolites from Medicinally Important Plant *Moringa oleifera*. International Journal of Pharmaceutical Sciences Review and Research 2011; 7(2): 186-188.
14. Sagwan S, Rao DV, Sharma RA: Biochemical Estimation of Primary Metabolites from *Pongamia pinnata* (L.): An Important Biodiesel Plant. International Journal of Pharmaceutical Sciences Review and Research 2010; 5(1): 146-149.
15. Singh S, Tanwer BS, Khan M: In Vivo and In Vitro Comparative Study of Primary Metabolites of *Commiphora wightii* (Arnott.) Bhandari. IJABPT. 2011; 2(1): 162-166.

16. Sagwan S, Rao DV, Sharma RA: Phytochemical evaluation and quantification of primary metabolites of *Maytenus emarginata* (Willd.) Ding Hou. J. Chem. Pharm. Res. 2010; 2(6): 46-50.
17. Sharma N, Tanwer BS, Vijayvergia R: Study of primary metabolites and antimicrobial activities of *Digera muricata* (L.) Mart. J. Chem. Pharm. Res. 2011; 3(2): 424-431.
18. Tewari JP, Shukla IK: Inhibition of infectivity of two strains of watermelon mosaic virus by latex of some angiosperms. Geobios. 1982; 9(3): 124-126.
19. Thomas OO: Re-examination of the antimicrobial activities of *Xylopia aethiopica*, *Carica papaya*, *Ocimium gratissimum* and *Jatropha curcas*. Fitoter. 1989; 60: 147-161.
20. Grosvenor PW, Supriono A, Gray DO: Medicinal Plants from Riau province, Sumatra, Indonesia. Part II: antibacterial and antifungal activity. J. Ethnopharmacol. 1995; 45: 97-111.
21. Mujumdar AM, Misar AV, Salaskar MV, Upadhye AS: Antidiarrhoeal effect of an isolated fraction (JC) of *Jatropha curcas* roots in mice. J. Nat. Remedies. 2001; 1: 89-93.
22. Gubitz GM, Mittelbach M, Trabi M: Exploitation of the tropical oil seed plant *Jatropha curcas* L. Bioresour. Technol. 1999; 67: 73-82.
23. Murashige T, Skoog F: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum 1962; 15: 473-497.
24. Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F: A colorimetric method for the determination of sugar. Nat. 1951; 168: 167.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin-Phenol Reagent. J. Biol. Chem. 1951; 193: 265–275.
26. Wong CK, Leung KN, Fung KP, Choy YM: Immunomodulatory and anti-tumour polysaccharides from medicinal plants. J. Int. Med. Res. 1994; 22(6): 299-312.
27. Lindhorst TK: Essentials of Carbohydrate Chemistry and Biochemistry. 2nd ed. Kiel, Germany: Wiley-VCH; 2003.
28. Freeze HH: Disorders in Protein Glycosylation and Protein Therapy. The J. Ped. 1998; 133(5): 593-600.
29. Tester RF, Karkalas J: The effects of environmental conditions on the structural features and physico-chemical properties of starches. Starch. 2001; 53(10): 513–519.
30. Griffin GJL, Wang JK: Industrial Uses. In: Wang JK, editors. Taro - a review of *Colocasia esculenta* and its potentials. Honolulu, USA: University of Hawaii press; 1983. p. 301-312.
31. Schwach E, Averous L: Starch based biodegradable blends: Morphology and interface properties. Polymer Inter. 2004; 53: 2115- 2124.
32. Entwistle G, Bachelor S, Booth E, Walker K: Economics of starch production in the UK. Industrial Crop Prod. 1998; 7(2-3): 175-186.
33. Thomsen S, Hansen HS, Nyman U: Ribosome-inhibiting proteins from in vitro cultures of *Phytolacca dodecandra*. Planta. Med. 1991; 57(3): 232-236.
34. Yadav PR, Tyagi R: Lipid Biotechnology. New Delhi, India: Discovery Publishing House Pvt. Ltd; 2006.
35. Harwood JL: Plant Lipid Metabolism. In: Dey PM, Harborne JB, editors. Plant Biochemistry. 3rd ed. California, USA: Academic Press; 2000. p. 237-271.
36. Hollman PCH: Evidence for health benefits of plant phenol: local or systemic effects? J. Sci. Food and Agr. 2001; 81(9): 842-852.