



Research Article

ESTIMATION OF PRIMARY AND SECONDARY METABOLITES FROM LEAVES OF THREE MEDICINAL PLANTSShikha Khandelwal^{1*}, Anirudh Rishi² and SM Paul Khurana¹¹Amity Institute of Biotechnology, Amity University Haryana, Manesar, Gurgaon, India²SP Institute of Biotech, Jaipur, Rajasthan, India

*Corresponding Author Email: writein.shikha@gmail.com

Article Received on: 10/08/14 Revised on: 20/09/14 Approved for publication: 12/10/14

DOI: 10.7897/2230-8407.0510159

ABSTRACT

Medicinal plants are important source of life saving drugs for majority of the world population. The study includes phytochemical screening and quantification of primary and secondary metabolites like chlorophyll, carbohydrates, protein, phenol, starch and ascorbic acid from leaves extract of selected plants namely: *Chenopodium album*, *Moringa oleifera* and *Terminalia arjuna*. The highest amount of protein (75.46 µg/ml) was observed in *T. arjuna*, ascorbic acid (38.02 mg/g dw), starch (5.12 mg/g dw), phenols (22.55 mg/g dw) and chlorophyll (14.75 mg/g dw) was observed in leaves of *C. album*.

Keywords: Primary metabolites, Protein, Chlorophyll, Ascorbic acid, medicinal plants, *Moringa oleifera*, *Chenopodium album*, *Terminalia arjuna*.

INTRODUCTION

Since ancient times, about 80 % of individuals use traditional medicine, which has chemical compounds derived from medicinal plants. These compounds are classified into primary and secondary metabolites¹. Primary metabolites are essentially required for growth and development of plants such as sugars, proteins, lipids and starch whereas chlorophyll, amino acids, nucleotides and carbohydrates have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. Secondary metabolites are not involved directly and they have been worked as biocatalysts which are synthesized during secondary metabolism of plants and are potential sources of drugs. The most important secondary metabolites are saponin, alkaloids, tannins, flavonoids and cardiac glycosides². Phytochemical screening is the technique to identify these compounds present in the plant extracts derived from any part of the plants like bark, leaves, flowers, seeds, etc. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency³⁻⁵. Many plants such as *Nerium indicum*, *Gloriosa superba*⁶, *Ricinus communis* and *Euphorbia hirta*⁷, *Pongamia pinnata*⁸, *Svensonia hyderabadensis*⁹ and *Melothria maderaspatana*¹⁰, *Cichorium intybus*¹¹, *Eclipta alba*¹², *Morinda citrifolia*¹², *Mangifera indica*¹³, *Cissus populnea*¹⁴ and *Bauhinia tomentosa*¹⁵ have been evaluated for their composition of primary metabolites and secondary metabolites for their medicinal values. In the present study investigations have been made to identify the medicinal properties of three plants: *Chenopodium album*, *Moringa oleifera* and *Terminalia arjuna* and their Qualitative phytochemical screening and estimation of primary and secondary metabolites will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds.

MATERIALS AND METHODS**Medicinal plants**

The medicinal plants under study were selected on the basis of review and ethno pharmacologic effects. Leaves of the following plants viz. *Chenopodium album*, *Moringa olerifera* and *Terminalia arjuna* were screened for the study.

Phytochemical Screening of extracts

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, phenols and tannins, steroids, saponins and cardiac glycosides was performed on the extracts as described by Trease and Evans¹⁶.

Quantitative Estimation of Primary and Secondary Metabolites

Quantification of primary and secondary metabolites was carried out by the following methods of carbohydrates¹⁷, chlorophylls¹⁸, proteins¹⁹, total phenols²⁰ and ascorbic acid²¹.

RESULTS AND DISCUSSION

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated²². In the present investigation primary and secondary metabolites were qualitatively and quantitatively analyzed in the three medicinal plants namely: *Chenopodium album*, *Moringa oleifera* and *Terminalia arjuna*. The results are presented in Table 1 and 2. In the present study the extract of *T. arjuna* showed maximum number of plant constituents such as flavonoids, phenol, tannins, steroids, glycosides, alkaloids and saponins, *M. oleifera* showed good presence of flavonoids and tannins whereas tannin was not found in *C. album* (Table 1). Our results were in agreement with findings of^{23,24}. The medicinal value of plants lies in some chemical substances that have definite physiological functions in the human body. Different phytochemicals have been found to possess a wide range of medicinal properties, which may help in protection against various diseases. For example, alkaloids protect against chronic diseases; saponins protect against

hypercholesterolemia and steroids and triterpenoids show the analgesic properties. The quantitative estimation of primary and secondary metabolites reveals various chemical constituents present in the plant (Table 2). Chlorophyll is the most indispensable class of primary compounds as they are the only substances that capture sunlight and make it available to plant system for its cultivation on photosynthesis²⁵. The chlorophyll content of *C. album*, *M. oleifera* and *T. arjuna* is 14.75 mg/g, 14.32 mg/g and 13.49 mg/g respectively. Ascorbic acid content was found high in *C. album* (38.02 mg/g dw) as it is not only an important antioxidant, it also appears to link flowering time, developmental senescence, programmed cell death and responses to pathogens through a complex signal transduction network^{26,27}. The extract of *C. album* showed higher level of phenols (22.55 mg/gdw) followed by *T. arjuna* and *M. oleifera*. The higher amount of phenol is important in regulation of plant growth, development and

disease resistance and it also possess various biochemical activities such as antioxidant, anti mutagenic, anti carcinogenic as well as ability to modify the gene expression²⁸. Carbohydrate content was found high in *M. oleifera* (6.11 mg/g dw). Proteins are the primary components of living organisms. The presence of higher protein levels in the plants increase food value or that a protein base bioactive compound could also be isolated in future²⁹. Protein content was found high in *T. arjuna* (75.46 µg/ml) followed by *M. oleifera* (54.65 µg/ml) and *C. album* (36.36 µg/ml). The same type of phytochemical screening of primary and secondary metabolites was reported earlier by many workers. The present investigation showed significant variation in the contents like phenol, flavonoids and tannin when compared to above mentioned reports. These variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned by³⁰.

Table 1: Preliminary phytochemical screening of crude extract of *Chenopodium album*, *Moringa oleifera* and *Terminalia arjuna* leaves

Group	Test	<i>Chenopodium album</i>	<i>Moringa oleifera</i>	<i>Terminalia arjuna</i>
Phenols	Lead acetate test	+	+	+
Alkaloids	Wagner's test	+	+	+++
Flavonoids	Shinoda test	+	++	++
Steroids	Salkowski's test	+	+	+++
Tannins	Ferric chloride test	-	++	++
Saponin	Frothing test	-	+	-
Cardiac glycosides	Keller-kiliani test	+	+	++

Table 2: Estimation of primary and secondary metabolites in leaves of *Chenopodium album*, *Moringa oleifera* and *Terminalia arjuna*

S. No	Primary Metabolites	<i>Chenopodium album</i>	<i>Moringa oleifera</i>	<i>Terminalia arjuna</i>
1	Carbohydrates (mg/g dw)	5.65	6.11	5.07
2	Chlorophyll (mg/g)	14.75233	14.32403	13.49101
3	Protein (µg/ml)	36.36	54.65	75.46
4	Phenols (mg/g dw)	22.55	20.82	21.32
5	Starch (mg/g dw)	5.12	4.58	4.92
6	Ascorbic acid (mg/g dw)	38.02	35.26	37.98

CONCLUSION

In the present study, the selected three plants have the potential to act as a source of useful drugs because they are the source of primary and secondary metabolites such as chlorophyll, protein, lipids, phenols, flavonoids and tannin. As medicinal plants play an important role in curing various diseases and antimicrobial, anticancer, antipyretic, astringent, antiviral activities of these plants are due to these compounds. Analysis of these metabolites is necessary for knowing the nutritional potential as well as helpful in manufacturing new drugs.

REFERENCES

- Vinoth S, Rajeshkanna P, Gurusaravanan P, Jayabalan N. Evaluation of phytochemical, Antimicrobial and GC-MS analysis of Extracts of *Indigo feratrita* L. f. Spp. *Subulata* (Vahl ex Poir). *Int J Agri Res* 2011; 6: 358-367. <http://dx.doi.org/10.3923/ijar.2011.358.367>
- Lingarao M, Savithramma N. Phytochemical studies of *Svensoniahyderabadensis* (Walp.) Mold – are medicinal plant. *Der Pharm Lett* 2011; 3: 51-55.
- Sasikala A, Linga RM, Savithramma N. Quantification of Primary and Secondary metabolites from leaves and stem bark of *Cochlospermum religiosum* (L) alston. *Int. Res. J. Pharm* 2013; 4(8): 228-231. <http://dx.doi.org/10.7897/2230-8407.04845>
- Gislene GF, Nascimento, Juliana Locatelli, Paulo C Freitas, Giuliana L Silva. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-Resistant Bacteria. *Braz. J. Microbiol* 2000; 31(4): 247-256.
- Ikram M, Inamul H. Screening of medicinal plants for antimicrobial activities. *Fitoterapia* 1984; 5: 62-64.
- Rishi A, Sarin R. Estimation of primary metabolites from *Gloriosa superb* L. *in vivo* and *in vitro*. *Int J Mendel* 2009; 26(1-4): 87.
- Vijayavergia R, Sharma S, Sing T. Biochemical estimation of primary metabolites of some medicinal plants of Euphorbiaceae family. *J Indian Bot Soc* 2009; 88: 116-119.
- Sagwan S, Rao DV, Sharma RA. Biochemical estimation of primary metabolites from *Pongamia pinnata* (L.): An important biodiesel plant. *Int J Pharma Sci Res* 2010; 5: 146-149.
- Lingarao M, Savithramma N. Quantification of primary and secondary metabolites of *Svensoniahyderabadensis* – a rare medicinal plant. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012; 4: 519-521.
- Choudhary S, Tanwer BS, Vijayavergia R, Singh T. Preliminary phytochemical screening and primary metabolites of *Melothria maderaspatana* (Linn.). *Cong International Journal of Biological and Pharmaceutical Research* 2013; 4(3): 168-171.
- Nandagopal S, Kumari BDR. Phytochemical and antibacterial studies of *Chicory* (*Cichorium intybus* L.) – A multipurpose medicinal plant. *Advan Biol Res* 2007; 1: 17-21.
- Sharma MC, Sharma S. Phytochemical screening of methanolic extract and antimicrobial activity of *Eclipta alba* and *Morinda citrifolia* L. *Middle-East J Sci Res Plants* 2010; 6(5): 445-449.
- Gupta C, Garg AP, Gupta S. Antimicrobial and phytochemical studies of fresh ripe pulp and dried unripe pulp of *Mangifera indica* (AMCHUR). *Middle-East J Sci Res* 2010; 5: 75-80.
- Boopathi CA, Sivakumar R. Phytochemical screening studies on the leaves and stem of *Andrographis neesianan* wight – An endemic medicinal plants from India. *World Appl Sci J* 201; 12(3): 307-311.
- Soladoye MO, Chukwuma EC. Quantitative phytochemical profile of the leaves of *Cissus populnea* Guill and Perr (Vitaceae) – An important medicinal plant in central Nigeria, *Archives of Applied Science Research* 2012; 4(1): 200-206.
- Trease GE, Evans WC. *Pharmacognosy*. Brailiar Tiridel can, 13th ed. Macmillian Publishers; 1989.
- Krishnaveni S, Balasubramanian T, Sadasivam S. Phenol Sulphuric acid Method, *Food Chemistry* 1984; 15: 229. [http://dx.doi.org/10.1016/0308-8146\(84\)90007-4](http://dx.doi.org/10.1016/0308-8146(84)90007-4)

18. Holden M. Chlorophylls. In chemistry and biochemistry of plant pigments. Goodwin TW (Ed.). Academic Press. London; 1960. p. 462-488.
19. Lowery OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
20. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of Drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem 2003; 51: 2144–2155. <http://dx.doi.org/10.1021/jf020444+>
21. Chinoy JJ. Formation and utilization of ascorbic acid in the shoot apex of Wheat as factor of growth and development. Ind J Plant Physiol 1962; 5: 172-201.
22. Ambasta SP, Ramachandran K, Kashyapa K, Chand R. Useful plants of India. Publication and information directorate. Council of Scientific and Industrial Research, New Delhi; 1986. p. 433–7.
23. Talreja T. Biochemical estimation of three primary metabolites from medicinally important plant *Moringa oleifera*. Int J Pharma Sci Rev Res 2011; 7: 186-188.
24. Sharma P. Phytochemical estimation of two primary metabolites from medicinally useful plant, *Terminalia arjuna* (Roxb.) Wt. and Arn. Current Biotica 2012; 6(3): 349-353.
25. Murray AP, Gibbs CF, Longmore AR. Determination of chlorophyll in marine waters: Inter comparison of a Rapid HPLC method with full HPLC, Spectrophotometric and Fluorometric methods. Marine chemistry 1986; 19: 211-227. [http://dx.doi.org/10.1016/0304-4203\(86\)90024-1](http://dx.doi.org/10.1016/0304-4203(86)90024-1)
26. Nicholas. The function and metabolism of ascorbic acid in plants. Annals of Botany 1996; 78: 661- 669. <http://dx.doi.org/10.1006/anbo.1996.0175>
27. Mapson LW. Metabolism of ascorbic acid system in plants. Part I. Function. Ann Rev Plant Physiol 1958; 2: 119-150. <http://dx.doi.org/10.1146/annurev.pp.09.060158.001003>
28. Nakamura Y, Watanabe S, Miyake N, Kohno H, Osawa T. Evaluation as novel radical scavenging antioxidants. J Agric Food Chem 2003; 51: 3309-3312. <http://dx.doi.org/10.1021/jf0341060>
29. Thomas S, Handen HS, NymnV. Ribosome inhibiting proteins from *in vitro* cultures of *Phytolacca decandra*. Plants Medica 1991; 57: 232-236. <http://dx.doi.org/10.1055/s-2006-960080>
30. Kokate CK, Purohit AP, Gokhale SB. Practical Pharmacognosy; 2nd edition. Vallabh Prakashan, New Delhi; 2004. p. 466-470.

Cite this article as:

Shikha Khandelwal, Anirudh Rishi and SM Paul Khurana. Estimation of primary and secondary metabolites from leaves of three medicinal plants. Int. Res. J. Pharm. 2014; 5(10):783-785 <http://dx.doi.org/10.7897/2230-8407.0510159>

Source of support: Nil, Conflict of interest: None Declared