Short Communication

Fluorescence analysis and extractive values of some Ethno-medicinal plants of Sikkim Himalaya region

Sonam Bhutia*

Government Pharmacy College, Government of Sikkim, Sikkim University, Sajong, Rumtek East Sikkim-737135, India

Received: 8 January 2020

Revised: 14 February 2020

Accepted: 16 February 2020

Abstract

Objective: Five ethnomedicinal plants from the Sikkim region, viz. *Ocimum sanctum*, *Azadirachta indica*, *Trigonella foenum-graecum*, *Tinospora cordifolia* and *Gymnema sylvestre* were considered in the present study. **Materials and Methods:** The present study will assist in standardization for quality, purity and sample identification. Here we mainly considered two standardization parameters like fluorescence activity and extraction yield respectively, the given medicinal plants were analysed using standard methods. **Results:** In the experimental analysis, we found that among extractive yield with different solvents, the aqueous extract displayed the highest yield followed respectively by ethanol. The fluorescence analysis for five Ethno-medicinal plants were conducted by using the visible light and ultra violet at 254nm and reveals the various colouration ranges from black to greenish brown colour with distinct morphological characteristics highlighted. **Conclusion:** The present study concludes that the data obtained can be used to authenticate, classify and standardize the above five ethno-medicinal plants. It was a first of its kind knowledge reported in a research paper.

Keywords: Fluorescence analysis, Traditional plants, extractive values, Sikkim Himalaya, Ethno-medicine plants

Introduction

The World Health Organization states about 80% of the population globally depends on traditional medicine. Moreover, use of plants in the United States is increasing significantly, to the phase that almost all pharmacies and supermarkets provide herbal products easily. Between 1990 to 1997, while complementary and alternative medicinal products increased from 34% to 42%, herbal consumption quadrupled from 3% to 12%. It is important to note that all these dramatic changes emerged not from medical profession but also from market pressure. The public has discovered that herbal medicines can create a safe, effective and economical option to pharmaceuticals, and this discovery is verified in science (Eisenberg et al., 1998). The lack of documentation and strict quality assurance procedures are barriers to the acceptance of alternative medicinal products in developing

countries. For the worldwide acceptance of this medicine scheme, the recording and standardization of the raw materials used in herbal medicine are thus very important. In identifying and authenticating the genuine plant products, pharmacognostic standardisation, physico-chemical examination and preliminary phytochemical studies were embraced. Right identification and quality control of plant materials is necessary to ensure the reproducible outcome of herbal medicinal products, which will lead to their health and effectiveness (Anonymous et al., 1998). Many primitive products come from wild sources and are typically obtained by weak, analphabet tribal people without much knowledge of botanical identification and authentication. Since of their heterogeneous nature the natural products are multifaceted the standardization is also a dynamic task. Appropriate regulation of starting material is extremely important for assessing the supremacy of herbal medicines. The primary speed for quality assurance of starting material is authentication, which can be followed by the development of numerical values for evaluation standards. Pharmacognostic criteria for unproblematic identification,

*Address for Corresponding Author:

Mr. Sonam Bhutia, Assistant Professor
Department of Pharmacognosy, Government of Sikkim, Sajong, East Sikkim- 737135 India
E-mail: sonamkzbhutia@gmail.com

DOI: <https://doi.org/10.31024/apj.2020.5.1.5>

2456-1436/Copyright © 2020, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

such as microscopy, physicochemical analysis, fluorescence analysis, are only a few of the essential labels for herbal standardization (Johansen et al., 1940).

Materials and methods

Collection and authentication of plant materials

The Ethno-medicinal plants: *Ocimum sanctum*, *Azadirachta indica*, *Trigonella foenum-graecum*, *Tinospora cordifo*, and *sylvestre Gymnema* were collected from the Sikkim Himalayan district during the duration of August-September 2018. Through scientific literature review or survey recognizes the individual plant.

Preparation of plant extracts

The collected crude drugs were washed completely with water to expel any undesirable material. These were additionally dried in shade. After complete drying of material, it was powdered for further processing (Handa et al., 2008; Rai et al., 2020).

Physiochemical evaluation

The different parameters in this aspect like ash values such as total ash, acid insoluble ash, sulphated ash and extractive values like water soluble extractives, alcohol soluble extractives were carried out as method described by Pharmacopoeia of India. Moisture content was carried out as method described by Khandelwal (2003). Determination of fluorescence analysis was followed as method described by Anonymous (1998).

Preparation of aqueous extract

The 20 gms of each powdered plant material was cold macerated with 100 ml of distilled water for 7 days. Additionally 5 drops of chloroform were added to it (Sandhya et al., 2010).

Drying of extract

The aqueous extract obtained from the process of extraction i.e. maceration was concentrated by using water bath. After complete evaporation, dried extract was stored in a well closed container to prevent the growth of moulds and microorganisms (Ransworth et al., 1966; Khandelwal et al., 2003).

Extractive value

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into the consideration, the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractives (Sharanabasappa et al., 2007; Singh et al., 2012).

Water soluble extract

Macerated 5 gm of the air dried coarsely leaves powder with 100 ml of chloroform water in a closed flask for 24 hours. Shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions

against loss of the solvent. Evaporated 25 ml of the filtrate to dryness in a tarred bottom flat bottom shallow dish dried at 105°C and weighed. The percentage of water soluble extractive value was calculated with reference to the air dried drug.

Alcohol soluble extract

Macerated 5 gm of the air dried coarsely leaves powder with 100 ml of 95 % ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat bottom shallow dish dried at 105°C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug.

Fluorescence analysis

The powdered drug and alcoholic extract was examined under U.V. and ordinary light with different reagents. About 10gm of the powdered drug was taken in a Petridis and treated with reagents viz, methanol, 50% sulphuric acid, 50% potassium hydroxide, 1N Hydrochloric acid, AgNO₃, 1N Sodium hydroxide. These were observed under different wavelengths i.e. visible rays and ultraviolet rays (254 nm and 365 nm) (Nagulan et al., 2016; Sudhakar et al., 2011).

Results and discussion

In the experimental evaluation, we found that among extractive yield with different solvents, the aqueous extract displayed the highest yield: 26.4% w/w for *Azadirachta indica* followed by ethanol: 12% w/w for *Gymnema sylvestre* respectively. Fluorescence analysis of the powder from the Ethno-medicinal plants were treated with required chemical reagents and observed under visible and ultra violet rays (254nm), reported in different colouration.

Table 1. Extractive value of *Ocimum sanctum*, *Azadirachta indica*, *Trigonella foenum-graecum*, *Tinospora cordifolia* and *Gymnema sylvestre*

Plant name	Extracts	Extractive value (% w/w)
<i>Ocimum sanctum</i>	i. Water soluble extracts	14.4%
	ii. Alcohol soluble extracts	6.4%
<i>Azadirachta indica</i>	i) Water soluble extracts	26.4%
	ii) Alcohol soluble extracts	7.2%
<i>Trigonella foenum-graecum</i>	i) Water soluble extracts	10.4%
	ii) Alcohol soluble extracts	7.2%
<i>Tinospora cordifolia</i>	i) Water soluble extracts	9.6%
	ii) Alcohol soluble extracts	2.4%
<i>Gymnema sylvestre</i>	i) Water soluble extracts	20.8%
	ii) Alcohol soluble extracts	12%

Fluorescence analysis of *Ocimum sanctum*: The selected plant was made in to courses powder and treated with following chemical reagents and observed under visible and ultra violet rays (254nm). The results are presented in table 2.

Fluorescence analysis of *Azadirachta indica*: The selected plant was made in to courses powder and treated with following chemical reagents and observed under visible and ultra violet rays (254nm). Results are shown in table 3.

Fluorescence analysis of *Trigonella foenum-graecum*: The selected plant was made in to courses powder and treated with

following chemical reagents and observed under visible and ultra violet rays (254nm). Results are shown in table 4.

Fluorescence analysis of *Tinospora cordifolia*: The selected plant was made in to courses powder and treated with following chemical reagents and observed under visible and ultra violet rays (254nm). Results are shown in table 5.

Fluorescence analysis of *Gymnema sylvestre*: The selected plant was made in to courses powder and treated with following chemical reagents and observed under visible and ultra violet rays (254nm). Results are shown in table 6.

Table 2. Fluorescence analysis of the *Ocimum sanctum* powder under visible and ultra violet rays

Treatment of powder	Visible rays	Ultraviolet light (254nm)
Powder as such	Greenish brown	Black
Powder + distilled water	Black	Black
Powder + H ₂ SO ₄	Greenish brown	Black
Powder + HCL	Brown	Black
Powder + 1N NaOH	Greenish brown	Deep black
Powder + methanol	Greenish black	Deep black
Powder + ethanol	Black	Deep black
Powder + glacial acetic acid	Greenish brown	Black

Table 3. Fluorescence analysis of the *Azadirachta indica* powder under visible and ultra violet rays

Treatment of powder	Visible rays	Ultraviolet light (254nm)
Powder as such	Green	Dark green
Powder + distilled water	Green	Black
Powder + H ₂ SO ₄	Light green	Dark green
Powder + HCL	Light green	Black
Powder + 1N NaOH	Dark green	Black
Powder + methanol	Black	Deep black
Powder + ethanol	Black	Deep black
Powder + glacial acetic acid	Green	Blackish green

Table 4. Fluorescence analysis of the *Trigonella foenum-graecum* powder under visible and ultra violet rays

Treatment of powder	Visible rays	Ultraviolet light (254nm)
Powder as such	Yellow	Greenish yellow
Powder + distilled water	Yellow	Greenish yellow
Powder + H ₂ SO ₄	Yellow	Green
Powder + HCL	Yellow	Dark green
Powder + 1N NaOH	Dark yellow	Dark green
Powder + methanol	Yellow	Green
Powder + ethanol	Yellow	Green
Powder + glacial acetic acid	Yellow	Dark green

Table 5. Fluorescence analysis of the *Tinospora cordifolia* powder under visible and ultra violet rays

Treatment of powder	Visible rays	Ultraviolet light (254nm)
Powder as such	Brown	Black
Powder + distilled water	Black	Black
Powder + H ₂ SO ₄	Black	Black
Powder + HCL	Black	Black
Powder + 1N NaOH	Black	Greenish black
Powder + methanol	Brown	Black
Powder + ethanol	Brown	Black
Powder + glacial acetic acid	Black	Black

Table 6. Fluorescence analysis of the *Gymnema sylvestre* powder under visible and ultra violet rays

Treatment of powder	Visible rays	Ultra violet light (254nm)
Powder as such	Light green	Dark green
Powder + distilled water	Light green	Dark green
Powder + H ₂ SO ₄	Light green	Dark green
Powder + HCL	Light green	Dark green
Powder + 1N NaOH	Yellowish green	Green
Powder + methanol	Dark green	Black
Powder + ethanol	Dark green	Black
Powder + glacial acetic acid	Brown	Black

Conclusion

World Health Organisation (WHO) has addressed its need to ensure quality checks of the materials used in identification and authentication of the medicinal plants through the use of modern techniques and the implementation of acceptable criteria and standards. In the present research, various standardization parameters such as fluorescence parameters and extractive value of the five Sikkim Himalayan ethno-medicinal plants were studied, which are first of its kind to record the knowledge and could be useful in the authentication and preparation of an effective monograph for proper identification of the leaves of the above medicinal plants for future reference.

Ethics approval and consent to participate

N/A

Funding

N/A

Acknowledgement

The authors are grateful to the host institute, Government Pharmacy College, Sajong to give such advanced facilities such as working facilities, instruments, chemicals, internet and

friendly environment, etc. to collect the significance information or data for this research work by providing the technical environment. Thanks to Entire college team, especially faculty members of GPC, Sajong for their continue support which helps me to overcome all the technical research queries during the data collection work.

Competing interest

The author has no conflict of interest to disclose.

References

- Anonymous. 1998. Macroscopic and Microscopic Examination: Quality Control Methods for Medicinal Plant Materials. Geneva: WHO.
- Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Rompay MV, Kessler RC, 1998. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. Journal of American Medical Association, 280:1569-1575.
- Handa SS, Khanuja SPS, Longo G, Rakesh DD. 2008 Extraction technologies for medicinal and aromatic plants. International Centre for Science and Higher Technology, 21-25.

- Johansen DA. 1940. Plant microtechnique. New York: Mc Graw Hill Book Co.
- Khandelwal KR. Practical Pharmacognosy. In: Preliminary Phytochemical Screening. 10th ed. Pune: Nirali Prakashan; 2003.
- Nagulan S, Kumar SR. 2016. Phytochemical, physicochemical and fluorescence analysis of leaf extract of *Syzygium calophyllifolium* Walp. Asian Journal Pharmaceutical and Clinical Research, 9(1):275-278.
- Rai B, Bhutia S, Pal P, Kakoti BB. 2020. Phytochemical analysis and antibacterial evaluation against selected gram strains by *Oroxylum indicum* (L.) Kurz stem bark extract, a folklore medicine of Sikkim Himalaya. Journal of Pharmacognosy and Phytochemistry, 9(1):11-16.
- Ransworth NR. 1966. Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences, 55:225-276.
- Sandhya S, Jaffery SAH, Vinod KR. 2010. Pharmacognostical studies on the leaf and root of *Physalis angulata* L. International Journal of Pharma Research and Development, 2(1): 001-008.
- Sharanabasappa GK, Santosh MK, Shaila D, Seetharam YN, Sanjeevrao I. 2007 Phytochemical studies on *Bauhinia racemosa* Larn. *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb. European Journal of Chemistry, 4:21-31.
- Singh SK. 2012. Phytochemical analysis of leaf callus of *Bacopa monnieri*. International Journal of Scientific and Research Publication, 2(9):1-3.
- Sudhakar A, Ramesh C, Nagaraju N, Murthy SRK. 2011. Pharmacognostical studies on the root tubers of *Pimpinella tirupatiensis* Bal. and Subr.-An endemic to Tirumala Hills of Eastern Ghats, India. International Journal of Pharmacognosy and Phytochemistry Research, 3(3):56-60.