Research Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

PHYSICOCHEMICAL AND PHYTOCHEMICAL EVALUATION OF *LEEA INDICA* (BURM.F.) MERR.

Sushmitha AR [1]*, Subrahmanya Padyana [2]

[1] PG Scholar, Department of Dravyaguna Vijnana, Alva's Ayurveda Medical College, Moodabidri, Dakshina Kannada, Karnataka, India

[2] Professor and HOD, Department of Dravyaguna Vijnana, Alva's Ayurveda Medical College, Moodabidri, Dakshina Kannada, Karnataka, India

Received on: 22/09/22 Accepted on: 25/10/22

***Corresponding author**
E-mail: drsushmithaar8@gmail.com

DOI: 10.7897/2277-4343.1306158

ABSTRACT

Leea indica (Burm.F.) Merr is a shrub or small tree which belongs to the family Vitaceae. It is used in the treatment of various diseases by traditional practitioners all over the world, like skin diseases, body pain, diarrhoea, dysentery, and snake bite etc. traditional practitioners of Dakshina Kannada district use *Leea indica* to treat skin diseases, body pain, diarrhoea, dysentery, snake bites, etc. The current paper is focused on the macroscopic, microscopic physicochemical and phytochemical studies of *Leea indica* root. Results of the prior quoted studies showed that *Leea indica* root is greenish brown with no characteristic odour and physicochemical studies showed moisture content of 50.44%, total ash value of 16.16% and pH of 5.56. Phytochemical studies revealed the presence of carbohydrates, proteins, saponins, tannins, and ellagic acid in Soxhlet and cold extraction with different solvents.

Keywords: *Leea indica*, Vitaceae, Root, Physicochemical study, phytochemical study

INTRODUCTION

According to World Health Organization (WHO), more than 80% of the population depends on traditional medicine for their primary healthcare needs. "Traditional medicine refers to health practices, approaches, knowledge, and beliefs incorporating plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain wellbeing". *Leea indica* (Burm.F.) Merr is one such drug used by traditional practitioners all over southern Asia to treat various ailments. *Leea indica* (Burm.F.) Merr belongs to the family Vitaceae and is used in treating various diseases by traditional practitioners worldwide, skin diseases, body pain, diarrhoea, dysentery, snake bites, etc[1]. Drugs have biological activities like antibacterial activity, antifungal activity, antiviral activity, anthelmintic activity, antimalarial activity, antioxidant activity, cytotoxic activity, anticancer activity, antidiabetic activity, wound healing activity, anti-inflammatory activity, CNS activity, hepatoprotective activity, antipyretic activity, anticonvulsant activity, thrombolytic activity, antiedematogenic activity etc[2]. An experimental study conducted using a Ferric ion-reducing antioxidant power assay showed the highest antioxidant capacities in methanol and ethanol extracts, and the methanol and ethanol leaf extracts were found to be selectively cytotoxic *in vitro* to (DU145 and PC-3) prostate cancer cell lines[3]. A cell line study showed ethyl acetate fraction (LIEAF) having the greatest cytotoxic effect against Ca Ski cervical cancer cells and inhibiting cervical cancer cell growth[4].

MATERIALS AND METHODS

Sample collection: The botanically identified samples of *Leea indica* (Burm. F.) Merr roots were collected from its natural habitat in and around Moodabidre, authenticated by Dr Subrahmanya Padyana, director of ATMA research centre Moodbidri, Karnataka, India. The collected root of the plant was appropriately cleaned, dried in the shade, made into coarse and fine powder separately, and stored in an airtight container.

Place of study: Physicochemical and phytochemical analysis was done in the Dravyaguna laboratory and Advanced research laboratory at Alva's Ayurveda medical college. Moodabidri, Karnataka, India.

Physicochemical Analysis

The drug in different forms was analysed for other physicochemical parameters like

- Determination of Moisture content
- Determination of Ash and its analysis
- Determination of pH
- Determination of Extractable substances[5,6]

Determination of moisture content (loss on drying)

The determination of moisture content helps in the identification and preservation of the drug and also the shelf life of the drug.

About 10 gm of 3 independent drug samples were placed in a tared evaporating dish after accurately weighing them. Dried at 105 ^{0}C for 1 hour and weighed. The drying and weighing continued at one-hour intervals until the difference between two successive weighings corresponded to less than 0.25%. Constant weight was reached when two consecutive weighs, after drying for 30 minutes and cooling for 20 minutes in a desiccator, showed not more than 0.1 gm difference.

Determination of ash

Three methods can determine the presence of ash value in the medicinal plant

- Total ash
- Acid insoluble ash
- Water-soluble ash

Six independent samples of 5 gm powdered drug were taken in a weighed silica crucible and incinerated at a temperature not exceeding 450 ^{0}C until free from carbon, cooled, and weighed. Calculate the percentage of ash concerning the air-dried drug.

Determination of water-soluble ash

Boil the ash for 5 minutes with 25 ml of water, collect insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450 ^{0}C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash concerning the air-dried drug.

Determination of acid-insoluble ash

Boil the ash obtained in total ash for 5 minutes with 25 ml of dilute hydrochloric acid, collect the insoluble matter on an ashless filter paper, wash with hot water, and ignite to constant weight. Calculate the percentage of acid-insoluble ash concerning the air-dried.

Ash analysis: Ash analysis is done for Carbonates, fluorides, Chlorides, Sulfate chromate, Phosphate, Potassium, Sodium, Aluminium, Calcium

Determination of pH value

A pH is a unit of measure which describes the degree of acidity or alkalinity of a solution. It is measured on a scale of 0 to 14. 100 mg of Soxhlet extract of the sample was dissolved in 100ml of distilled water. Calibration of the pH meter was done using a standard buffer solution. pH was calculated by immersing the glass electrode of the pH meter in the sample. The constant value in the pH was tabulated.

Table 1: Results of Moisture lost at each hour

Time	Sample 1		Sample 2		Sample 3	
	Dry weight in gm	Moisture lost in gm	Dry weight in gm	Moisture lost in gm	Dry weight in gm	Moisture lost in gm
	10	0	10	0	10	0
1st hour	5.611	4.389	5.611	4.389	5.611	4.389
2nd hour	4.516	5.484	5.090	4.911	5.653	4.347
3rd hour	4.484	5.516	5.035	4.965	5.595	4.405
17th hour	4.503	5.497	5.055	4.495	5.611	4.389
18th hour	4.450	5.55	4.993	5.007	5.5511	4.449
19th hour	4.445	5.555	4.976	5.024	5.527	4.473
20th hour	4.438	5.562	4.968	5.032	5.525	4.475
21st hour	4.412	5.588	4.958	5.042	5.523	4.477
22nd hour	4.410	5.59	4.957	5.043	5.509	4.491
23rd hour	4.406	5.594	4.956	5.044	5.507	4.493
24th hour	4.406	5.594	4.955	5.045	5.056	4.494

Table 2: Results of Ash value

Sample	Sample weight	Ash weight
Sample 1	5 gm	0.154
Sample 2	5 gm	0.128
Sample 3	5 gm	0.213
Sample 4	5 gm	0.114
Sample 5	5 gm	0.175
Sample 6	5 gm	0.146

Table 3: Results of ash analysis

Ash analysis for	Water-soluble ash	Acid insoluble ash
Carbonates	Absent	Present
Fluorides	Absent	Absent
Chlorides	Absent	Absent
Sulfate	Absent	Absent
Chromate	Absent	Absent
Phosphate	Absent	Absent
Potassium	Absent	Absent
Sodium	Present	Absent
Aluminium	Absent	Present
Calcium	Absent	Present

Table 4: Results of extractive value in Soxhlet extraction in distilled water

Sample	Sample weight in gm	Extract weight in gm
Sample 1	30	2.088
Sample 2	30	3.356
Sample 3	30	2.712

Table 5: Results of extractive value in cold extraction with distilled water

Sample	Sample weight in gm	Extract weight in gm
Sample 1	10	0.453
Sample 2	10	0.45
Sample 3	10	0.378

Table 6: Results of extractive value in cold extraction with methanol

Sample	Sample weight in gm	Extract weight in gm
Sample 1	10	1.05
Sample 2	10	0.828
Sample 3	10	0.641

Table 7: Results of extractive value in cold extraction with ethanol

Sample	Sample weight in gm	Extract weight in gm
Sample 1	10	0.962
Sample 2	10	0.871
Sample 3	10	0.94

Table 8: Results of Phytochemical studies in distilled water in Soxhlet extraction

Test names	Sample 1	Sample 2	Sample 3
Carbohydrates			
Benedict's test	Absent	Present	Present
Fehling's test	Present	Present	Present
Molisch's test	Present	Present	Present
Protein			
Biuret's test	Absent	Present	Present
Mallon's test	Absent	Present	Present
Starch	Absent	Absent	Absent
Alkaloids			
Dragendorff's test	Absent	Absent	Absent
Mayer's test	Absent	Absent	Absent
Flavonoids	Present	Present	Present
Ellagic acid	Present	Present	Present
Saponins	Present	Present	Present
Resins	Present	Present	Present
Steroids			
Salkowski reaction	Present	Present	Present

Table 9: Results of Phytochemical studies in distilled water in cold extraction

Test name	Sample 1	Sample 2	Sample 3
Carbohydrates			
Benedict's test	Present	Present	Present
Fehling's test	Present	Present	Present
Molisch's test	Absent	Absent	Absent
Protein			
Biuret's test	Present	Present	Present
Mallon's test	Present	Present	Present
Starch	Absent	Absent	Absent
Alkaloids			
Dragendorff's test	Absent	Absent	Absent
Mayer's test	Absent	Absent	Absent
Flavonoids	Absent	Absent	Absent
Ellagic acid	Present	Present	Present
Saponins	Absent	Absent	Absent
Resins	Absent	Absent	Absent
Steroids			
Salkowski reaction	Present	Present	Present

Table 10: Results of phytochemical studies in Methanol in cold extraction

Test name	Sample 1	Sample 2	Sample 3
Carbohydrates			
Benedict's test	Present	Present	Present
Fehling's test	Present	Present	Present
Molisch's test	Absent	Absent	Absent
Protein			
Biuret's test	Present	Present	Present
Mallon's test	Present	Present	Present
Starch	Absent	Absent	Absent
Alkaloids			
Dragendorff's test	Absent	Absent	Absent
Mayer's test	Absent	Absent	Absent
Flavonoids	Present	Present	Present
Phenolics	Present	Present	Present
Ellagic acid	Absent	Absent	Absent
Tannins	Absent	Absent	Absent
Saponins	Absent	Absent	Absent
Resins	Absent	Absent	Absent
Steroids			
Salkowski reaction	Present	Present	Present

Table 11: Results of phytochemical studies in Ethanol in cold extraction

Test name	Sample 1	Sample 2	Sample 3
Carbohydrates			
Benedict's test	Present	Present	Present
Fehling's test	Present	Present	Present
Molisch's test	Present	Present	Present
Protein			
Biuret's test	Present	Present	Present
Mallon's test	Present	Present	Present
Starch	Absent	Absent	Absent
Alkaloids			
Dragendorff's test	Absent	Absent	Absent
Mayer's test	Present	Present	Present
Flavonoids	Absent	Absent	Absent
Ellagic acid	Absent	Absent	Absent
Tannins	Absent	Absent	Absent
Saponins	Present	Present	Present
Resins	Absent	Absent	Absent
Steroids			
Salkowski reaction	Present	Present	Present

Determination of extractable matter

This method determines the number of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which, as yet no suitable chemical or biological assay exists. About 10 gm of coarsely powdered air-dried material of the trial drug is accurately weighed and placed in a glass-stoppered conical flask and macerated with 100 ml of specified solvent for 6 hours by shaking frequently and then allowed to stand for 18 hours. It was filtered rapidly, taking care not to lose any solvent and evaporated to dryness in a water bath. Dried at 105 ^{0}C for 6 hours, cooled in a desiccator for 30 minutes, and weighed without delay. The amount of extractable matter in mg/gm of trail drug was calculated. The amount of extractable matter was determined for Alcohol soluble extractive and water-soluble extractive using respective solvents. According to standard protocol, the qualitative analysis is done for carbohydrates, proteins, tannins, saponins, flavonoids, phenols, steroids, alkaloids, and starch resins.

OBSERVATION AND RESULTS

Determination of moisture content
The percentage of moisture content of the drug *Leea indica* is 50.44%. (Table 1)

Determination of total ash
Percentage of total ash of the drug *Leea indica* is 16.16%. (Table 2)

Ash analysis: Determination of pH value
The pH value of *Leea indica* is 5.56. (Table 3)

Determination of Extractive Value: Soxhlet extraction
The percentage of the extractive value of *Leea indica* in Soxhlet extraction is 9.06%. (Table 4)

Cold extraction: Distilled water
The percentage of the extractive value of *Leea indica* in distilled water is 4.27%. (Table 5)

Cold extraction: Methanol
The percentage of the extractive value of *Leea indica* in methanol extraction is 8.396%. (Table 6)

Cold extraction: Ethanol
The percentage of the extractive value of *Leea indica* in ethanol extraction is 9.243%. (Table 7)

Preliminary Phytochemical Study

Soxhlet extraction: Distilled water (Table 8)
Cold extraction: Distilled water (Table 9)
Cold extraction: Methanol (Table 10)
Cold extraction: Ethanol (Table 11)

DISCUSSION

A physicochemical study revealed that the moisture content is 50.44% because the sample was taken in the wet form for analysis. Total Ash 16.16%, water-soluble ash 0.854%, insoluble acid ash 3.66% high acid insoluble ash value may be because the drug consists of aluminium and calcium. The pH value of *Leea indica* is 5.66, showing the drug is mildly acidic. Soxhlet extract of *Leea indica* (Burm. F.) Merr in distilled water is 9.06%. Extract of the drug in cold maceration method with distilled water, methanol and ethanol are 4.27%, 8.39% and 9.2 %, respectively. The extractive value of the drug is maximum in ethanol; hence it can be considered as the drug contains ethanol-soluble phytochemicals in large numbers.

Preliminary phytochemical study of *Leea indica* (Burm. F.) Merr root showed the presence of carbohydrates, proteins, phenolics, flavonoids, Gallo tannins, ellagic acid saponins, resins and steroids in Soxhlet extraction with distilled water, carbohydrates, proteins, Gallo tannins, ellagic acid and steroids in distilled water, carbohydrates, proteins, phenolics and steroids in methanol extract and ethanol extract in cold maceration method, carbohydrates, proteins, flavonoids and steroids are found. Studies on Anti-inflammatory properties of flavonoids: human studies proved that flavonoids affect inflammatory biomarkers[6]. Tannins are known to have antibacterial activities[7]. Previous studies have shown that Saponins have anticancer and anticholesterol activity[8]

CONCLUSION

The moisture content of the drug is 50.44%, Total Ash 16.16%, water-soluble ash 0.854%, acid insoluble Ash 3.66%. The drug is mildly acidic with a pH of 5.56. The extractive value of the drug is maximum in ethanol at 9.2%. In the phytochemical study of *Leea indica,* root carbohydrates, proteins, phenolics, flavonoids, gallotannins, ellagic acid saponins, resins, and steroids are present.

REFERENCES

1. Souravh Bais. A Phytopharmacological Review on an Important Medicinal Plant: *Leea indica*. Inventi Rapid: Ethnopharmacology, 2013;(1): 1-4, 2013.
2. Farhad Hossain Md, Golram Mostofa, AHM Khurshid Alam. Traditional uses and pharmacological activities of the genus leea and its phytochemicals: A review. Heliyon, 2020.e06222.
3. Shridhar C. Ghagane, Sridevi I. Puranik, Vijay M. Kumbar, Rajendra B. Nerli, Sunil S. Jalalpurec, Murigendra B. Hirematha, Shivayogeeswar Neelagund, Ravindranath Aladakatti. *In vitro* antioxidant and anticancer activity of *Leea indica* leaf extracts on human prostate cancer cell lines, Integrative Medicine Research, 2017;6(1): 79-87.
4. Wong Yau Hsiung and Habsah Abdul Kadir, *Leea indica* Ethyl Acetate Fraction Induces Growth-Inhibitory Effect in Various Cancer Cell Lines and Apoptosis in Ca Ski Human Cervical Epidermoid Carcinoma Cells, Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine. 2011, Article ID 293060, 13, DOI:10.1155/2011/293060.
5. API, Part 2, Vol 1, The controller of publications, Ministry of Health; FW, Dept of AYUSH, 2004, P 159, 160
6. Xinyu Zhao, Ruyi Chen, Yueyue Shi, Xiaoxi Zhang, Chongmei Tian and Daozong Xia; Antioxidant and Anti-Inflammatory Activities of Six Flavonoids from *Smilax glabra* Roxb, Molecules 2020;25:5295 DOI:10.3390/ molecules25225295 www.mdpi.com/journal/molecules.
7. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: A review. Crit Rev Food Sci Nutr. 1998 Aug;38(6):421-64. DOI: 10.1080/10408699891274273.
8. Güçlü-Ustündağ O, Mazza G. Saponins: properties, applications and processing. Crit Rev Food Sci Nutr. 2007;47(3):231-58. DOI: 10.1080/10408390600698197.

Cite this article as:

Sushmitha AR and Subrahmanya Padyana. Physicochemical and Phytochemical evaluation of *Leea indica* (Burm.F.) Merr. Int. J. Res. Ayurveda Pharm. 2022;13(6):66-70 http://dx.doi.org/10.7897/2277-4343.1306158

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