Journal of Stress Physiology & Biochemistry, Vol. 19, No. 3, 2023, pp. 79-86 ISSN 1997-0838
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ORIGINAL ARTICLE

OPEN ACCESS

Quantitative Analysis of Physicochemical and Phytochemical Constituents Medicinal Herbs

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Received March 2, 2023

The present investigation has been carried out the Stastical Analysis of physicochemicaland phytochemical constituents medicinal herbs such as flower of *Calendula officinalis* Linn, leaves of *Lantana camara* Linn,aerial part of *Oldenlantia herbacea* L and root of *Peristrophe paniculata* Forssk.In physicochemical analysis, parameters such as foreign matter, loss drying,total ash,water and acid soluble ash, water and solvent extractive value of plant were determined.And also the quantitative estimation of phytochemical constituents like alkaloids, tannin, terpenoids, saponin, carbohydrates and protein contents of *Calendula officinalis* L., *Lantana camara* L., *Oldenlandia herbacea* (L.) Roxb., and *Peristrophe paniculata* Forssk. was undertaken as per standardized procedure.Result showed that Loss on drying turned out to be 1.70% in *Calendula officinalis* L.,1.61 % in *Lantana camara* L.,1.45% in *Peristrophe paniculata* Forssk. and 1.31% in *Oldenlandia herbacea* Roxb. Foreign matters were found to be 2.36% (*Calendula officinalis* L.), 2.67% (*Lantana camara* L.), 4.03% (*Peristrophe paniculata* Forssk.) and 3.73% in *Oldenlandia herbacea* (L.) Roxb and the toal ash was found to be relatively 9.46% in *Calendula officinalis* L. followed by 10.62 % in *Lantana camara* L., 9.53% in *Peristrophe paniculata* Forssk. and 8.34% in *Oldenlandia herbacea* Roxb. The water solubility of all the selected plants 21.74% (*C. officinalis*), 22.43% (*L. camara*), 22.58% (*P. paniculata*) and 22.68% (*O. herbacea*) were found to be higher compared to ethanol and other solvents. The phenol content was higher in *Lantana camara* L. (15.95mg) followed by *Calendula officinalis* L. (12.57mg), *Peristrophe paniculata* Forssk. (10.63mg) and *Oldenlandia herbacea* (L.) Roxb. (3.70mg). The high content of alkaloid was recorded in *Calendula officinalis* L. (12.57mg) and low content in the *Peristrophe paniculata* Forssk. (0.39mg). The total tannin content of *Calendula officinalis* L. (78.93µg) was found to be higher than that of other plants.The total flavonoid content of *Calendula officinalis* L. was 21.51mg followed by 16.44mg for *Lantana camara* L., 21.29 mg for *Peristrophe paniculata* Forssk. and 18.43 mg for *Oldenlandia herbacea* (L.) Roxb. *Oldenlandia herbacea* (L.) Roxb. contains higher concentration of terpenoids (11.65mg/g) compared than that of other three plants. Among the four plants, the levels of carbohydrate and protein content were found to be higher in *Calendula officinalis* L.(61mg; 5.20mg) respectively. From th results it was concluded that this study will be helpful in standardization for quality, purity and sample identification.

Key words: Calendula officinalis L., phytochemicals, Foreign matter, Oldenlandia herbacea (L.) Roxb.

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. Plants have been used extensively as medicine for the treatment of various ailments throughout human history and even today this trend continues. According to the World Health Organization (W.H.O), approximately 75-80% of the world's population use plant-based medicines. All plants may not be as useful as claimed, or may have more therapeutic properties than are known traditionally. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plants (Khan *et al*., 2006). World Health Organization recommends promotion of traditional/herbal remedies in National Health care programme because such drugs are easily available at low cost, are comparatively safe and the people have faith in such remedies. Plant materials and herbal remedies derived from them represent a substantial promotion of the global drug market and in this respect internationally recognized guidelines for their quality assessment are necessary. Adulterations and substitution have become a major problem to the absence of standards relating to genuineness of drugs, skill hands and cost factors, etc. The standardization of crude drugs can be achieved by stepwise Pharmacognostic and physicochemical studies (Niveditha Devi *et al*., 2012).

Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs.

Phytochemicals are biologically active naturally occurring chemical compounds which are found in plants (Hasler and Blumberg, 1999). Their main role is to protect the plants from disease and damage caused by abiotic and biotic stresses and these chemicals also contribute to the plant's aroma, colour and flavour. These plant chemicals i.e. phytochemicals, are produced to protect plant cells from environmental hazards such as drought, stress, pollution, pathogenic attack and UV exposure (Mathai, 2000). Currently they have an imperative role in maintaining the well-being of humans without any diseases so they ultimately enhance the quality of life.

Oldenlantia herbacea Roxb. belonging to Rubiaceae family, is an erect , glabrous annual herb found in temperate and tropical regions. The aerial part of this plant used for wound healing. Ethnomedically, this plant is recognized as one of the ingredient in Jaundice treatment. Decoction of the herb can be used for the treatment of rheumatic arthritis and swellings. *Calendula officinalis* Linn. or pot marigold is a common plant belonging to Asteraceae family , native to southern Europe. The plant species has been reported to contain a variety of phyto-chemicals, including carbohydrates, phenolic compounds, lipids, steroids, tocopherols, terpenoids, quinones and carotenoids (Kishimoto *et al*., 2005). *Lantana camara* Linn. (Verbenaceae Family) is a low erect or subscandent vigorous shrub with tetrangular stem, stout recurved pickles and a strong odour of black currents.It was used to treat cuts, rheumatisms, ulcers, catarrhal infection, tetanus, rheumatism, malaria, cancer, chicken pox, asthma, ulcer, swelling, eczema, tumour, high blood pressure, bilious fever, ataxy of abdominal viscera, sores, measles, fevers, cold and high blood pressure (Kirtikar *et al*., 2007). *Peristrophe paniculata* Forssk. Belonging to Acanthaceae family. The root is bitter, astringent, cooling and is useful in intermittent fever, intrinsic haemorrhage, ulcer, wounds, skin diseases, pruritus, worms, dental caries, leucorrhoea and insomnia.

MATERIALS AND METHODS

Identification and authentication

The plant species selected for the present study (*Calendula officinalis* Linn, *Lantana camara* Linn, *Oldenlantia herbacea* L. and *Peristrophe paniculata* Forssk.) were collected in and around trichy, identified with the help of Flora of Presidency of Madras (Gamble 1997) and authenticated with the specimen deposited at

RAPINAT Herbarium, St. Joseph's College, Trichy. **(Voucher specimen number:** BISH0000619230; BISH0000699120; S002; PARC/2012/1290).

Physicochemical Analysis

Tests for Identity, Purity and Strength (Anonymous , 2001)

Determination of Foreign Matter

100 g of the dry plant powder was weighed and separated out in a thin layer. The foreign matter was detected by inspection with the unaided eye. It was separated, weighed and calculated the percentage of foreign matter present.

Determination of Moisture Content (Loss on Drying)

10g of dry powder (without preliminary drying) was weighed accurately in a tarred evaporating dish. Care was taken so that no appreciable amount of moisture is lost during preparation and that was representative of the official sample. The weighed sample of the drug was placed in the tarred evaporating dish, dried at 105ºC for 5 hours, and weighed. The drying and weighing were continued at one-hour interval until difference between two successive weighing almost correspond and difference was not more than 0.25 %. When Constant weight was reached, the material was cooled for 30 minutes in a desiccator. The percentage of moisture content was calculated.

Determination of Total Ash

About 2g of accurately weighed dry plant powder was incinerated in a silica dish at temperature not exceeding 450ºC until free from carbon and weighed. The percentage of ash with reference to the air-dried drug was calculated.

Determination of Acid Insoluble Ash

The ash obtained from total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. Collected the insoluble matter in an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of ash with reference to the air-dried drug was calculated.

Determination of Water Soluble Ash

The ash obtained from total ash was bolied for 5 minutes with 25 ml of distilled water. Collected the insoluble matter in an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450ºC the weight of the insoluble matter subtracted from the weight of the ash, the difference in weight represents the water soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated.

Determination of Successive extractive values

Suitably weighed quantity of the air dried, crushed drug transferred to an extraction thimble, extracted with various solvents in the order of increasing polarity by using Soxhlet extraction apparatus (for 6 hours). Filtered the extract quantitatively into a tarred evaporating dish and evaporated off the solvent on a water bath. The residue dried at 105ºC to constant weight. The percentage of extractive values with reference to the air-dried drug, for various solvents was calculated.

Determination of Alcohol Soluble Extractive

5g of the air-dried plant powder was macerated with 100 ml of alcohol in a closed flask for 24 hrs frequently shaken during first 6hrs and allowed to stand for 18 hrs. After incubation period, the content was rapidly filtered, taking precautions against loss of solvents. Evaporated 25 ml of filtrate to dryness in a tarred flat bottomed china dish and dried at 105ºC until constant weight is obtained. The percentage of alcohol soluble extractive with reference to the air-dried drug was calculated.

Determination of Water Soluble Extractive

5g of the air-dried dry powder macerated with 100 ml of water in a closed flask for 24 hrs frequently shaken during first 6hrs and allowed to stand for 18 hrs. Rapidly filtered and evaporated 25 ml of filtrate to dryness in tarred flat-bottomed china dish and dried at 105ºC until constant weight is obtained. The percentage of water-soluble extractive with reference to the air-dried drug was calculated.

QUANTITATIVE ESTIMATION OF THE MAJOR METABOLITES

Estimation of phenols (Malick *et al.,* 2008)

1 gm of the sample was weighed and ground well with 10 times the volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min.

The supernatant was collected and the residue was re-extracted with 5 times the volume of 80% ethanol. The sample was centrifuged again and the supernatant was collected. It was then evaporated to dryness. The residue obtained was dissolved in 5 ml of distilled water. 0.5 ml of the sample was pipetted out in a test tubes, and the volume was made upto 3 ml with distilled water. 0.5 ml of Folin's reagent was added after 3 min 2 ml of 20% Sodium bicarbonate solution was added. The content was mixed thoroughly and the test tube was placed in boiling water bath for 1 min, cooled and the colour developed was measured at 650 nm.

Estimation of total flavonoids (Kadifkova Panovska *et al.,* 2008)

The ethyl acetate extracts were dried over anhydrous sodium sulphate, filtered and concentrated in boiling water bath upto a concentration of 1g/ml of extract. They were further diluted with ethyl acetate to obtain 0.01g/ml solution used for experiments. About 10ml of the solution was transferred into a 25ml volumetric flask, 1ml of 2% aluminium chloride was added and the solution was filled to a volume with methanol-acetic acid and was kept aside for 30 min, the absorbance was measured at 390nm. A blank was also maintained. Quercetin was used to construct the calibration curve in the concentration range of 1 to10µg/ml.

Estimation of total alkaloids (Ferguson 1956)

The alcoholic extract of plant sample was treated with 0.1N HCl and aqueous acidified layer thus obtained was partitioned with Chloroform in a separating funnel. The Chloroform layer was discarded and the aqueous layer was basified with Ammonium hydroxide to alkaline pH and partitioned with Chloroform in a separating funnel. The aqueous layer was discarded and the Chloroform layer was evaporated, the resultant content was treated as total alkaloid and confirmed for alkaloid with dragendroff's reagent.

Estimation of saponin (Hiai *et al.*, 1976)

0.5 ml plant sample and different aliquots of standard were taken in test tubes. 2 ml of 80% of ethanol and 2ml of 72 % of Sulphuric acid was added mixed well. Test tubes were incubated in boiling water at 60'c for few minutes. After incubation the absorbance was measured at 544 nm against reagent blank. Test value was calculated with standard value. The results were expressed as mg / 100 g of Saponin content, calculated using the following equation.

Determination of tannin (Price and Butter 1997)

0.1ml of test sample and different aliquots of standard solution of tannic acid were taken in a series of test tubes. A control was also maintained without sample. The volume was made up to 4.0 ml with distilled water. 2.0 ml of 10% of Sodium carbonate and 0.5 ml of Folin's Ciocalteau. The developed color was read at 620 nm. The total tannic content was expressed as mg of tannic equivalent per gram of plant powder. The results were expressed as mg / 100 g of tannin content, calculated using the following equation.

Extraction of carbohydrate – Anthrone method (Yemm and Willis, 1954)

About 2gm of the sample was homogenized with 20ml of 80% (v/v) of ethanol in water and the combined extract was made upto 100ml with distilled water in a volumetric flask. The extract was allowed to evaporated in a water bath and subsequently the sample was treated with 1ml of saturated lead acetate, again which was filtered and the filtrate was collected in to a beaker containing 3ml of saturated disodium hydrogen phosphate which was filtered and the filtrate collected in a beaker and was made up to 50ml in standard flask.

Estimation of carbohydrate – Anthrone method

About 0.1 ml of test solution was taken in a test tube. The volume was made up to 1ml with distilled water. About 4ml of 0.2% Anthrone was added to the test tube. The test tubes were incubated in ice bath. Various standards were taken in a series of test tube (0.2, 0.4, 0.6….). The tubes were heated in a boiling water bath for 10 minutes. The colour developed was read calorimetrically at 640nm

Extraction of protein

About 1gm of the sample was weighed and the sample was treated with 3ml of water. Add 5ml of 10% TCA which was kept for about 30 minutes. The contents were centrifuged and the supernatant was discarded.

The precipitate was dissolved in 3ml of 0.1NaOH and made up the volume with distilled water.

Estimation of protein (Lowry *et al*., 1958)

Into a series of test tubes various concentrations of standard protein solution were taken and the volume was made up to 1ml with distilled water. A blank was maintained with 1ml of water. For the test 0.1ml of sample was taken. Add 4.5ml of Lowry's reagent t all the tubes and allowed to stand for 10 minutes. After incubatio, 0.5ml of Folin's reagent was added to all the tubes and again incubated for 20minutes at room temperature. The blue colour developed was read calorimetrically at 620nm.

RESULTS AND DISCUSSION

Determination of loss on drying, foreign matter and ash value

The physicochemical standards are used to standardize the identity ,quality and purity of the drug. The physicochemical standards which are studied in the present study are loss on drying , foreign matter , ash value and extractive values. The results of the physicochemical standards of selected plants were shown in the Table 1.

Foreign matters were found to be 2.36% (*Calendula officinalis* L.), 2.67% (*Lantana camara* L.),4.03% (*Peristrophe paniculata* Forssk.) and 3.73% in *Oldenlandia herbacea* (L.) Roxb. The low levels of the foreign matter may be due to first hand collection of plant material from non polluted area (Khandelwal, 1998).

Loss on drying turned out to be 1.70% in *Calendula officinalis* L., 1.61% in *Lantana camara* L.,1.45% in *Peristrophe paniculata* Forssk. and 1.31% in *Oldenlandia herbacea* Roxb. which is not too high, hence it could be discourage the bacterial, fungal or yeast growth during storage (African Pharmacopoeia, 1986).

Ash value is useful for authenticity and purity of the drugs and also these values are important for quantitative standards. In the present study ,the toal ash was found to be relatively 9.46% in *Calendula officinalis* L. followed by 10.62 % in *Lantana camara* L.,9.53% in *Peristrophe paniculata* Forssk. and 8.34% in *Oldenlandia herbacea* Roxb., which may be due to the presence of carbonates, phosphates, silica and silicates. Water soluble ash was higher in *Calendula officinalis* L. (8.51%) compared than that of other three plants . Acid insoluble ash was higher in *Oldenlandia herbacea* Roxb. (1.67%) and lower in *Calendula officinalis* L. which is compared with other two plants. The results of the present study revealed the higher value of total ash, lesser amount of acid insluble ash which indicates the purity and presence of higher amount of inorganic contents in the plant sources.

Determination of extractive value

Estimation of extractive values determines the amount of active constituents in a given amount of plant materials when extracted with a particular solvent. The extraction of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depends upon the nature of the drugs and the solvent used. It also gives an indication whether the crude drug is exhausted or not. In the present study, the hexane – 2.45% (*C. officinalis*), 4.34% (*L. camara*), 1.64% (*P. paniculata*) and 2.98 % (*O. herbacea*) and chloroform - 5.03% (*C. officinalis*), 5.18% (*L. camara*), 6.60% (*P. paniculata*) and 5.60% (*O. herbacea*) extractive values are less when compared to ethyl acetate - 6.70% (*C. officinalis*), 7.05% (*L. camara*), 8.61% (*P. paniculata*) and 7.44% (*O. herbacea*)**.**

The water solubility of all the selected plants - 21.74% (*C. officinalis*), 22.43% (*L. camara*), 22.58% (*P. paniculata*) and 22.68 % (*O. herbacea*) - were found to be higher compared to ethanol and other solvents, which indicates the presence of highly polar chemical constituents such as flavonoids, proteins, carbohydrates, etc. Alcohol soluble extractive values were found to be 13.12%, 14.66%, 15.22% and 12.95% respectively which indicated the presence of polar constituents (Table 2).

Quantitative analysis of phytoconstituents

Phytochemical quantification is very important in identifying therapeuctically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, tannins and ascorbic acids. In the present study, the quantitative estimation of alkaloids, tannin,

terpenoids, saponin, carbohydrates and protein contents of *Calendula officinalis* L., *Lantana camara* L., *Oldenlandia herbacea* (L.) Roxb., and *Peristrophe paniculata* Forssk. was undertaken as per standardized procedure and the results have been reported in the Table 3 and Figure 1a & b.

The phenol content was higher in *Lantana camara* L. (15.95mg) followed by *Calendula officinalis* L. (12.57mg), *Peristrophe paniculata* Forssk. (10.63mg) and *Oldenlandia herbacea* (L.) Roxb. (3.70mg). The high content of alkaloid was recorded in *Calendula officinalis* L. (12.57mg) and low content in the *Peristrophe paniculata* Forssk. (0.39mg). The total tannin content of *Calendula officinalis* L. (78.93µg) was found to be higher than that of other plants.The total flavonoid content of *Calendula officinalis* L. was 21.51mg followed by 16.44mg for *Lantana camara* L., 21.29 mg for *Peristrophe paniculata* Forssk. and 18.43 mg for *Oldenlandia herbacea* (L.) Roxb. *Oldenlandia herbacea* (L.) Roxb. contains higher concentration of terpenoids (11.65mg/g) compared than that of other three plants. Among the four plants, the levels of carbohydrate and protein content were found to be higher in *Calendula officinalis* L. (61mg; 5.20mg) respectively.

From the above results, it was revealed the all those plants contains considerable number of secondary metabolites which is responsible for the diverse therapeutic effects especially antioxidant, antimicrobial, anti-inflammatory and wound healing etc. Tannin has been reported to possessing antiviral, antibacterial, and antitumor activity. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine), antibacterial (e.g., chelerythrine) , and antihyperglycemic activities (e.g., piperine). Flavonoids have been reported to possess a wide variety of biological activities among which are antimicrobial, anti-inflammatory, antiangiogenic, analgesic, antiallergic effects, cytostatic and antioxidant, antiviral, anticarcinogenic, anticancer as well as anti-diarrheal properties (Middleton, 2000). Terpenoids are known to possess a wide range of biological activities including antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, antihyperglycemic, anti-inflammatory, immunomodulatory properties.

Table 1. Determination of loss on drying, foreign matter and ash value

Name of the plants	Loss on Drying (% w/w)	Foreign matter (% w/w)	Total Ash (%w/w)	Water Soluble Ash (%w/w)	Acid Insoluble Ash (%w/w)
***Calendula officinalis* L.**	**1.70±0.09**	**2.36±0.13**	**9.46±0.26**	**8.51±0.35**	**1.25±0.20**
***Lantana camara* L.**	**1.61±0.20**	**2.67±0.29**	**10.62±0.40**	**7.73±0.25**	**1.68±0.25**
***Peristrophe paniculata*(Forssk.)**	**1.45±0.20**	**4.03±0.56**	**9.53±0.19**	**6.93±0.16**	**1.45±0.11**
***Oldenlandia herbacea* (L.) Roxb.**	**1.31±0.17**	**3.73±0.33**	**10.87±0.20**	**8.34±0.50**	**1.67±0.15**

Values expressed as Mean ± SE (n=3)

Table 2. Determination of extractive value

Parameters	*Calendula officinalis* L.	*Lantana camara* L.	*Peristrophe paniculata* (Forssk.)	*Oldenlantia herbacea* L. (Roxb.)
Hexane	**2.45±0.16**	**4.34±0.48**	**1.64±0.20**	**2.98±0.42**
Chloroform	**5.03±0.19**	**5.18±0.26**	**6.60±0.43**	**5.60±0.70**
Ethyl Acetate	**6.70±0.13**	**7.05±0.10**	**8.61±0.48**	**7.44±0.40**
Ethanol	**13.12±0.42**	**14.66±0.51**	**15.22±0.59**	**12.95±0.78**
Water	**21.74±0.49**	**22.43±0.36**	**22.58±0.42**	**22.68±1.12**

Values are expressed as Mean ± SE (n=3)

Table 3. Quantitative analysis of phytoconstituents

Parameters	*Calendula officinalis* L.	*Lantana camera* L.	*Peristrophe paniculata* (Forssk.)	*Oldenlandia herbacea* (L.) Roxb.
Alkaloids (mg/g)	3.79±0.17	0.56±0.06	0.39±0.02	0.55±0.04
Flavonoids (mg/g)	21.51±0.54	16.44±2.00	21.29±0.58	18.43±0.78
Phenol (mg/g)	12.57±0.50	15.95±0.66	10.63±0.27	12.19±0.57
Saponin (µg/g)	196.28±3.58	292.67±6.02	300.55±6.78	263.15±2.62
Tannin (µg/g)	78.93±1.74	60.67±2.42	20.91±1.07	31.99±0.72
Terpenoids (mg/g)	4.48±0.62	5.96±0.12	10.58±0.52	11.65±0.68
Carbohydrates (mg/g)	61.00±3.13	55.70±1.80	45.95±1.92	35.71±1.25
Proteins (mg/g)	5.20±0.25	2.54±0.37	3.74±0.27	2.30±0.30

Values are expressed as Mean ±SE

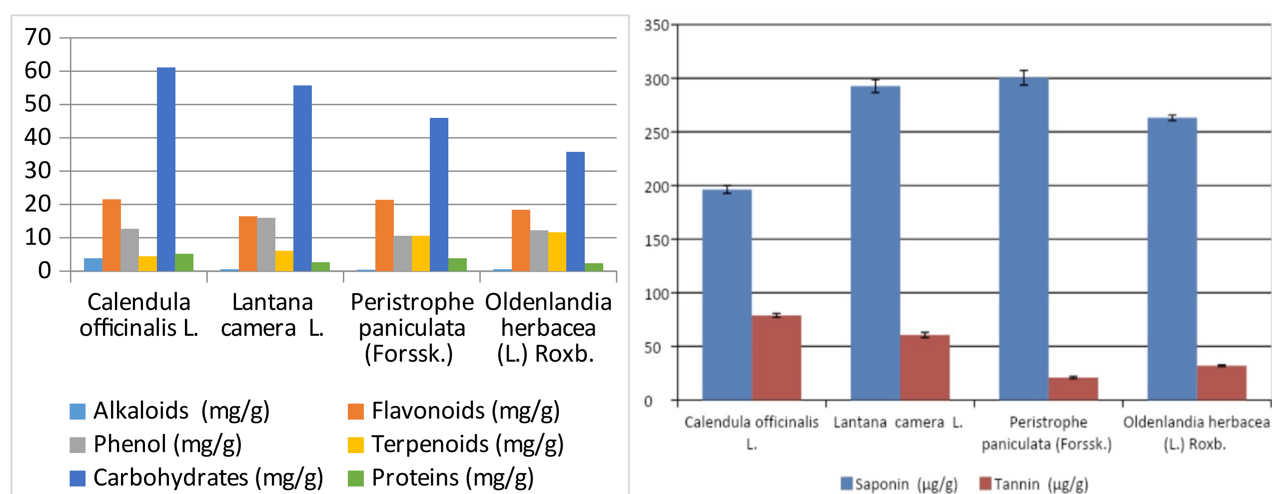

Figure 1. A & B. Quantitative analysis of secondary metabolites.

CONCLUSION

Medicinal Plants have been playing important role in curing the diseases of human being since time immemorial. The medicinal value of plants is due to some chemically active substances that produce a definite physiological action on the human body. Various Physicochemical parameters such as, total ash, acid insoluble ash, water soluble ash, water soluble extractive and alcohol soluble extractive value were observed. These values can be useful todetect adulteration. All studied standardization parameters like phytochemical screening and physicochemical parameters provide the knowledge in the identification authentication of selected plants. The phytochemical screening confirmed the presence of diverse phytochemical constituents such as alkaloids, flavonoids, amino acids, carbohydrate, proteins, saponin and tannins. Phytochemical constituents confirmed utilization of rhizome for treating diabetes, abdominal pains, menstrual disorder, wounds, eczema, Jaundice, inflammations and as a blood purifying activity.The present study could be used as a diagnostic tool for the standardization. Preliminary phytochemical screening suggests that they are responsible for pharmacological activities.

CONFLICTS OF INTEREST

The author declare that he has no potential conflicts of interest.

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