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Determination of Total Alkaloids and Tannins Contents in Leaves of Buchanania lanzan

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Prabhat Jatav
Department of Botany, Government Motilal Vigyan Mahavidyalaya, 462008, Bhopal (M.P.), India Barkatullah University, Bhopal, 462026 (M.P.), India

Rajesh Kumar Tenguria
Department of Botany, Government Motilal Vigyan Mahavidyalaya, 462008, Bhopal (M.P.), India Barkatullah University, Bhopal, 462026 (M.P.), India

Correspondence author email Id:
prabhatjatav.94@gmail.com

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Abstract
Qualitative analysis is very essential to identify the phyto constituents present in medicinal plants. The objective of this research was to investigate the phyto-constituents present in 5 diverse extracts of Buchanania lanzan leaves. Quantitative assessment was performed to quantify components such as alkaloids and tannins by the standard method. The present study suggested that the methanol and aqueous extracts included alkaloids, steroids, saponins, phenols and terpenoids. In addition, coumarin was also present in dichloromethane and aqueous extracts. High alkaloids and tannin contents were recorded in methanolic extract of the leaves. Colchicine was used as a standard for alkaloids and tannic acid for tannins. This study provides evidence that the leaves of Buchanania lanzan are of maximum therapeutic efficacy with the mainstream phytochemical classes of compounds. Whereas methanol is good solvents for the extraction of these type of phytochemicals. The results this confirm its therapeutic application in traditional medicine.

1. Introduction

The remedial importance of plants lies in definite chemical substances that create a certain physiological function on the human. The most crucial of these bioactive components are alkaloids, carbohydrates, flavonoids, phenols, saponins, proteins and tannins. Many indigenous medicinal plants are used as spices and food plants [1]. Plant chemicals are non-nutritive chemicals that are produced naturally in plants during metabolic processes and have a variety of activating properties or disease-preventive properties [2].

Alkaloids are category of low molecular weight amino acid-derived nitrogen-containing organic compounds, predominantly contained in a variety of organisms for example bacteria, fungi, plants and animals [3]. Alkaloids are secondary metabolites produced by plants in response to environmental modulation and biotic or abiotic stress. And alkaloids have structural diversity and vital natural activities. [4]. In nature, they are not only produced against herbivores but also reduce bacterial or fungal infections. Therefore, they are substances that have high potential in medicine, plant protection and toxicology [5].

Tannins are polyphenols usually occur in terrestrial and several marine plants. These tannins can be split into hydrolyzed and condensed tannins. [6]. Antioxidant properties of tannins are broadly used in food and remedial zones. Tannins have

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antibacterial, antiviral, antiparasitic, anti-inflammatory and antidiarrheal activity [7].

B. lanzan (Anacardiaceae family) is called Char in Hindi and Almondette in English. This plant is found in the deciduous tropical parts of India and its height up to 13-17 meter. The leaves are alternate, petiolate and very coriaceous [8]. Conventionally, leaves have been used for wound control and also as a digestive, expectorant and purgative [9]**.** Number of glycolipids has been isolated from this plant and other compounds reported are cardanol, cardol, anacardic acid and fatty acids [10].

2. Materials And Methods

Plant identification:

Plant identification and authentication was done from Minor Forest Produce Processing and Research Centre, Bhopal (M.P.) India and Vedanta Testing and Research Laboratory, Bhopal (M.P.) India.

Sample collection:

Leaves were selected for this study. Leaves of *B. lanzan* were collected from Ratapani forest area of Sehore district (M.P.). Collected leaves were brought to the testing room by placing them in polythene. The collected leaves were brought to the laboratory by placing them in polythene bags. And then it was thoroughly washed with detergent and water. The leaves were then dried in the shade area. After the leaves were dried, a grinder machine was used to make the powder and stored for further investigation.

Successive extraction:

The powdered material was subjected to a hot soxlation extraction method, sequentially in increasing order of polarity, of various known solvents using petroleum ether, ethyl acetate, dichloromethane (DCM), methanol and water. Sample extracts were concentrated by evaporation of the solvent on water bath at 80^{o}C for 4-5 days. All extracts were stored in the refrigerator at 10^{o}C for qualitative analysis [11].

Preliminary phytochemical analysis:

Different qualitative tests were carried out to establish absence or presence of phytochemicals. Preliminary phytochemical analysis was performed using standard procedures [12, 13].

Alkaloids:

The plant extract was taken in a glass tube and then a few drops of Mayer's reagent were added. Yellow or white creamy PPT indicates the presence of alkaloids because $K2HgI_4$ (potassium mercuric iodine) is present in reagent.

Amino acid:

05 ml of samples and few drops of 40% NaOH were dissolved. Then, few drops of 10% lead acetate were added. Black PPT indicates the positive test of amino acids.

Carbohydrates:

2-3 ml of the test sample and a few drops of α-naphthol were added and shaken vigorously. One ml of conc. H_2SO_4 was added gradually beside the inner surface of glass tube. Appearance of a violet ring between the two layers indicates the presence of this test.

Coumarins:

Two ml of test samples were mixed with three ml of 10% NaOH and few drops of chloroform were added. Formation of yellow color indicates the presence of coumarins.

Flavonoids:

One milliliter of crude sample was added to the tube and a few drops of 10% solution of lead acetate were added. It gives yellow color PPT when flavonoids are present.

Glycosides:

2 ml of extract was placed in glass tubes and a few drops of glacial acetic acid were added. And a drop of ferric chloride solution was added to it. Few drops of conc. sulfuric acid were added. In the presence of this test a reddish-brown ring is formed at the edge.

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Lignins:

Few drops of 5% gallic acid were added to a 1-2 ml test sample. Olive green color indicates the presence of lignin.

Oils and Fat:

Few amount of extracts were spread on whatman filter paper, formation of oil spots on the paper indicates the presence of oils and fat in the test samples.

Phenolic compound:

2-3 ml samples were taken in tubes. Few drops of 5% solution of ferric chloride were added. Formation of deep blue black color indicates the presence of phenolic compounds.

Protein:

2 ml of samples and few drops of Millon's solution were mixed. Appearance of white PPT which turns red on slight heating confirms the presence of this test.

Quinones:

Few drops of concentrated hydrochloric acid were mixed with two ml test samples. Green color indicates the occurrence of quinones.

Saponins:

Sample was dissolved into five ml of water (D/W). After addition of D/W it was shaken for accurate mixing till foam was observed. Stable foam indicates the occurrence of saponins.

Starch:

A few drops of dilute iodine solution were mixed with three ml of extract. The blue color is a positive test for the presence of starch.

Steroid:

Two ml of conc. sulphuric acid and two ml chloroform were mixed into 2 ml sample and shaken fine. Chloroform layer appears red and acid layer greenish yellow Fluorescence**,** which indicates the occurrence of steroid.

Tannins:

Few drops of 1% gelatin solution and NaCl (10%) were added to 1-2 ml of the sample. Formation of white PPT confirms the presence of tannins.

Terpenoid:

Five ml sample was mixed with two ml chloroform and evaporated on water bath, then conc. H_2SO_4 (3 ml) was added carefully. A gray colored solution indicates a positive test for terpenoid.

Total alkaloids contents (TAC):

Solution preparation

Solutions were prepared with 500 µl of 2 N HCl, 2.5 ml bromocresol green with 2.5 ml phosphate buffer (6.8 pH). The standard solution (colchicine) was made by adding ten mg of pure colchicine to ten ml of distilled water, and the plant extract (100 mg/1 ml) was prepared.

Preparation of standard curve

For accurately measure (20, 40, 60, 80 and 100 µg/ml) of colchicine standard solution and mid value of sample (60 µg) were taken and transfer into test tubes and volume makeup upto 1 ml with D/W. Then, 500 µl of HCl, 2.5 ml of BCG (bromocresol green) reagent and 2.5 ml phosphate buffer were mixed. The reagent was stirred well through vigorous shaking with two ml of chloroform, and placed in ten ml flask for settle down and diluted by Distilled water. After settle down the top layer was taken for absorbance. The absorbance of complex in chloroform was measured with a spectrophotometer (visible spectrophotometer) at 470 nm. Blank reagent was prepared as above but without the addition of sample or standard. Total alkaloids were expressed as µg/ml of sample [14].

Total tannins content (TTC):

The tannin content in the leaves was determined by the method presented by Price and Butler, (1977) [15].

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Solution preparation

0.008 Mole solution of Fecl3 was prepared in 0.008 N HCl. And 100 mg / 1 ml extracts and standard solutions of tannic acid 1 mg/ml were prepared. Then, a 0.003 mole solution of potassium ferrocyanide was prepared in distilled water.

Preparation of standard curve

60 µg of extract and tannic acid such as (100, 80, 60, 40 and 20 µg/ml) were taken in each glass tubes. Then, 1 ml $Fecl_3$ solution and 1 ml potassium ferrocyanide were mixed to every tube. Absorbance was taken at 720 nm and tannins content was expressed in µg/ml.

3. Result

Percentage yield:

The leaf extract obtained by Soxhlet was calculated for the percentage yield (%). The percentage amounts of pet ether, ethyl acetate, dichloromethane (DCM), methanol and aqueous leaf extracts obtained were 1.55%, 4.00%, 1.56%, 18.51% and 11.13% respectively.

Phytochemical analysis:

Preliminary phytochemical analysis for the leaves of *B. lanzan* is tabulated in Table 1. Qualitative tests were carried out by diverse chemical assays to identify the presence or absence of phyto contents like alkaloids, flavonoids, coumarins, glycosides, phenols and quinones etc.

Table 1: Screening of phytochemical from leaves of B. lanzan

Phyto Constituents	Petroleum ether	Ethyl acetate	Dichloro-methane	Methanol	Water
Alkaloids	-	+	-	+	+
Amino acid	-	-	-	-	+
Carbohydrates	-	-	-	-	-
Coumarins	-	-	+	-	+
Flavonoids	-	+	+	+	-
Glycosides	-	+	-	+	-
Lignins	-	-	-	+	-
Oils and Fat	+	-	+	+	-
Phenols	-	+	+	+	+
Proteins	-	-	+	+	-
Quinones	-	-	+	+	-
Saponins	-	-	+	+	+
Starch	-	-	-	-	-

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Steroid	-	-	-	+	+
Tannins	-	+	+	+	-
Terpenoids	-	-	-	+	+

+ Present, - Absent.

Total alkaloids content:

The amount of alkaloids found in the leaf extract was determined using colchicine. Results are shown in Table 2. All samples used in quantification were those that showed the presence of alkaloids tested in phytochemical screening using Meyer's reagent (fig. 1 and 2).

Table 2: Total alkaloid content of various extracts of leaves

Extract	Total Alkaloids (µg/ml)
Ethyl acetate	26.028
Water	20.366
Methanol	65.66

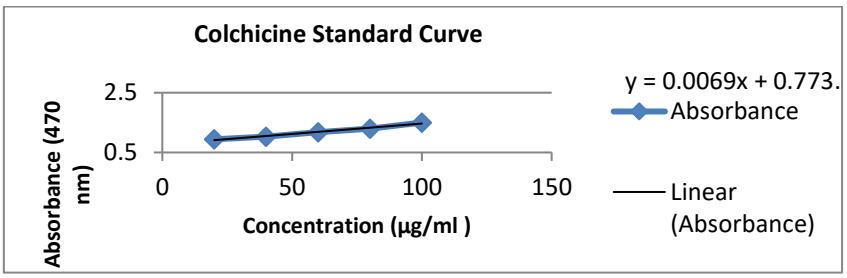

Figure 1: Standard curve of colchicine

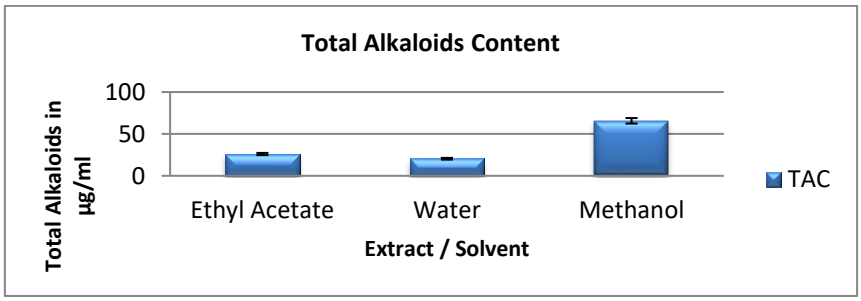

Figure 2: Amounts of alkaloids in µg/ml

Total tannins content:

B. lanzan various TTC values were found in leaves. And show significant differences in the total tannin content of the leaves (Table 3). Tannic acid was used as a standard for this protocol. (fig. 3 and 4).

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Table 3: Total tannins content of various extracts of leaves

Extract	Total tannins (µg/ml)
Ethyl acetate	11.708
Dichloromethane	21.405
Methanol	60.98

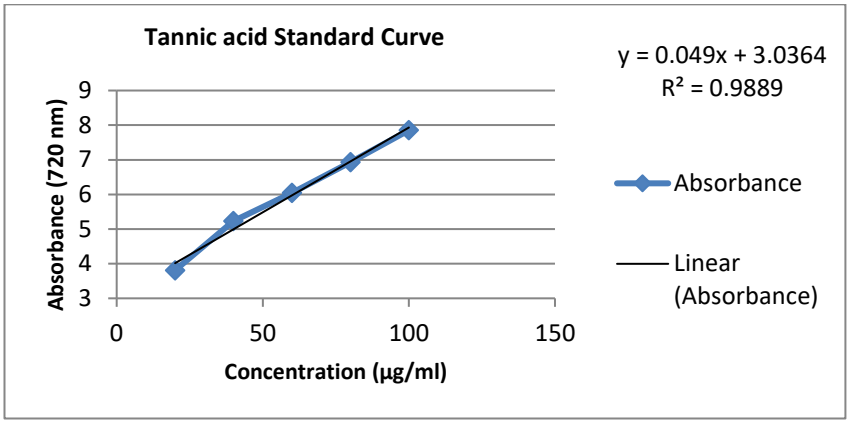

Figure 3: Standard curve of tannic acid

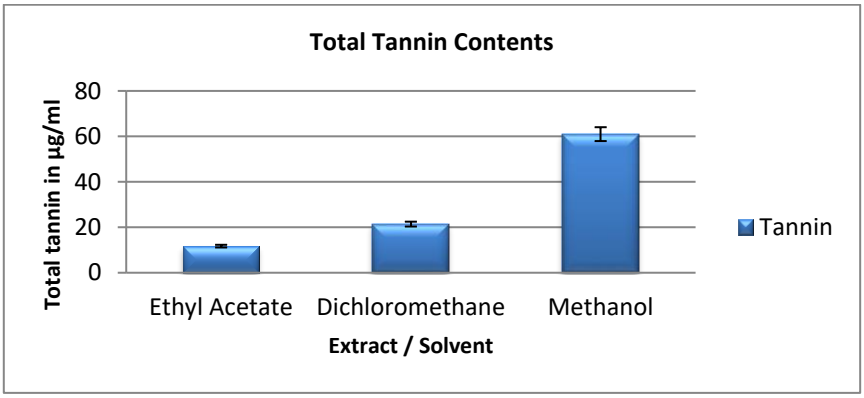

Figure 4: Amounts of tannins in µg/ml

4. Discussion

Plant derived materials have gained a lot of interest in human life nowadays. Solvents generally used in soxhlet extraction of plants are polar solvent (alcohols and H_2O), middle polar (acetone and DCM), and non-polar (n-hexane, ether and chloroform) [16]. Successive extraction was performed by the Soxhlet method using five different solvents. Which included methanol and water as the polar and petroleum ether as the non-polar solvent. The highest mass was obtained from 18.51% methanolic extract and the lowest mass was obtained from 1.55 % petroleum ether and 1.56% DCM. Need for phytochemical analysis has become essential, as many plants accumulate biologically active substances in various parts and tissues [17]. Alkaloids, flavanoids, saponins, tannins, triterpenes, steroids and quinones are

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famous due to significant biological action attributed to this category of compounds [18]. The presence of this phyto content ranged from abundant to poor or absent in most of the extracts analyzed. The plant *B. lanzan* give positive test of oils and fat in petroleum ether and alkaloids, flavonoids, glycosides, phenols and tannins in ethyl acetate extracts. The dichloromethane contains coumarins, flavonoids, oils and fat, phenols, proteins, quinones, saponins, tannins and methanol extract contains alkaloids, flavonoids, glycosides, lignins, oils and fat, phenols, proteins, quinones, saponins, steroid, tannins and terpenoids. Aqueous extract were found presence of alkaloids, amino acid, coumarins, phenols, saponins, steroid and terpenoids. Over the past few years, diversity of natural alkaloids prepared from plants or herbs have attracted substantial attention due to their superb anti-inflammatory and antioxidant activity [19]. Determination of alkaloids using the spectrophotometric method with BCG (bromocresol green) is an easy and highly sensitive technique that does not require any special equipment. BCG can react with some alkaloids, that is, those that contain nitrogen inside their structure, but not with amine and amide [20]. *B. lanzan* leaves shows 65.66 µg/ml high alkaloid content in methanolic extract. And 26.028 µg/ml in ethyl acetate while 20.366 µg/ml in aqueous extract. Tannins are called bactericidal because they react irreversibly with proteins, thus inactivating their activity by complexing within the bacterial membrane. Tannin-based pharmaceuticals have been used to treat intestinal infections. Several experimental studies have been published on the pharmaceutical utilize of tannins through anti-tumor and anti- oncogenic actions [21]. Their anti-viral efficiency has been also fine known by *in vitro* analysis for a diversity of twelve diverse hydrolysable and condensed tannins [22]. Ethyl acetate, dichloromethane and methanol extracts of leaves show 11.708, 21.405 and 60.98 µg/ml of total tannins, respectively.

5. Conclusion

The utility of remedial plants is a conventional practice in human life. It is of utmost importance to scientifically estimate the therapeutic use of plants and to give information about species that can be used for their properties in the prospect. In this study, the highest percentage yield was recorded in the methanol extract. Most of the phyto contents were present in the methanolic extract of *B. lanzan* leaf. Higher total alkaloids and tannin contents were observed in methanolic extracts 65.66 and 60.98 µg/ml respectively. Whereas the aqueous extract had less alkaloids (20.366 µg/ml) and ethyl acetate (11.708 µg/ml) had less tannin. The results obtained from the whole study confirm the presence of phyto-contents of *B. lanzan* leaves and suggest that the plant parts contain phyto- contents with extracts from special solvents. The extracts of the leaves can be used for novel formulations and patent medicines of natural source.

6. Abbreviations

DCM: Dichloromethane; BCG: Bromocresol Green; PPT: Precipitation; TAC: Total Alkaloid Content; TTC: Total Tannin Content; nm: Nanometer; µg/ml: Microgram per milliliter.

References

[1] Gujjeti RP, Mamidala E. Phytochemical screening and thin layer chromatographic studies of *Aerva lanata* root extract. Int J of Innovative Res in Sci Eng and Tech 2013; 2(10): 5725-30.

[2] Minakshi B, Jharna D, Chanbi DE, Nayan T, Partha PK, Kundal N, Manash PS. Phytochemical analysis of traditional medicinal plants and their antimicrobial activity: An experience from North East India. J of Pharma Res 2016; 1(1): 1-7.

[3] Moreira R, Pereira D, Valentao P, Andrade P. Pyrrolizidine alkaloids: Chemistry pharmacology toxicology and food safety. Int J of Mol Sci 2018; 19(1668):1-22.

[4] Taha HS, El Bahr MK, Seif MM. In vitro studies on Egyptian catharanthus roseus (L.) Ii Effect of biotic and abiotic stress on indole alkaloids production. J of Appl Sci Res 2009; 5(10) 1826-31.

[5] Adamski Z , Blythe LL, Milella L, Bufo SA. Biological activities of alkaloids: From

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

toxicology to pharmacology. Toxins 2020; 12(210); 1-4.

[6] Haslam EJ. Plant polyphenols: Vegetable tannins revisited (1989). Cambridge: Cambridge University Press; 1989.

[7] Tong Z, He W, Fan X, Guo A. Biological function of plant tannin and its application in animal health. Front in Veteri Sci 2022; 8:1-7. doi: 10.3389/fvets.2021.803657.

[8] Kodati D, Pareta S, Patra KC. Antiulcer activity of ethanolic extract of *Buchanania lanzan* Spreg. roots. Ann of Biol Res 2010; 1(4): 234-39.

[9] Kirtikar RK, Basu DB. Indian Medicinal Plants. Vol 3. Dehradun, India: International Book Distributor; 1935.

[10] Mehta SK, Mukherjee S, Jaiprakash B. Preliminary phytochemical investigation on leaves of *Buchanania lanzan* (Chironji). Int J Pharm Sci Rev Res 2010; 3(2):55-9.

[11] Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. United Nations Industrial Development Organization and the International Centre for Science and High Technology; 2008.

[12] Kokate CK. Practical pharmacognosy. 14th reprint ed. Delhi India: Vallabh Prakashan; 2010.

[13] Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. Inter J of Chem Stud. 2020; 8(2): 603-08.

[14] Shamsa F, Monsef H, Ghamooshi R, Rizi MV. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci 2008; 32: 17-20.

[15] Price ML, Butler LG. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J of Agric Food Chem 1977; 25(6): 1268-73.

[16] Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J of Pharm Bioall Sci 2020;12:1-10.

[17] Muniyandi JM, Lakshman K. Preliminary studies of phytochemical investigation on coastal medicinal plants of Boloor, Mangalore. Indo Americ J of Pharmaceu Sci. 2018; 05(02):1309-15.

[18] Maria R, Shirley M, Xavier C, Jaime S, David V, Rosa S, Jodie D. Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from *Guayas province* Ecuador. J of King Saud Uni Sci 2018; 30 500-05.

[19] Peng J, Zheng TT, Li X, Liang Y, Wang LJ, Huang YC, Xiao HT. Plant derived Alkaloids: The promising disease-modifying agents for inflammatory bowel disease. Front Pharmacol 2019; 10(351):1-15.

[20] Salamah N, Ningsih DS. Total alkaloid content in various fractions of *Tabernaemonata sphaerocarpa* Bl. (Jembirit) leaves. IOP Conf Series: Materi Sci and Eng 2017; 259:1-6. doi:10.1088/1757-899X/259/1/012017.

[21] Pizzi A. Tannins: Prospectives and actual industrial applications. Biomolecules 2019; 9(344): 1-30.

[22] 22. Kashiwada Y, Nonaka G, Nishioka I, Chang JJ, Lee KH. Antitumor agents, 129 tannins and related compounds as selective cytotoxic agents. J of Natu Prod 1992; 55(8): 1033-43.