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Research Article

Pharmacognostical Standardization of the Stem of *Vachellia jacquemontii* (Benth.) Ali

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Abstract

Vachellia jacquemontii (Benth.) Ali commonly known as *Acacia jacquemontii* and *Jacquemont's acacia* is locally to the Thar Desert of India and Pakistan. This herb has been used traditionally for a variety of medical conditions, such as chronic renal disease, rheumatism, joint discomfort, snakebite, fever and abortion induction. *Vachellia jacquemontii* (Benth.) Ali's stem is important because of its rich phytochemical profile, which calls for a careful Pharmacognostical analysis. Numerous pharmacological and biological characteristics, including anti-inflammatory, anti-bacterial, antihypertensive, antiplatelet, hypoglycemic, anti-atherosclerotic, anticancer, analgesic, free radical scavenging, and antioxidant qualities, are well-known for *Vachellia jacquemontii* (Benth.) Ali. The complex phytochemical profile of *Vachellia jacquemontii* depends on its stem, which calls for a careful analysis. Pharmacognostical standardization of the stem of *Vachellia jacquemontii* is the aim of this investigation.

Studies of the *Vachellia jacquemontii* stem's morphology and histology are part of the standardization process. The plant's physiochemical analyses, which included measurements of its ash value, moisture content, and pH, were in line with pharmacopoeial standards and reports. These findings show that the medicine is of adequate quality and satisfies all recommended parameters. Proteins, alkaloids, amino acids, saponins, flavonoids, and other phenolic compounds were found in the plant extracts after phytochemical screening; glycosides, sterols, carbohydrates and volatile oils were not found.

EEVJ had a high content of total flavonoids (191.09 ± 1.232 quercetin equivalents mg/g of EEVJ) and a low content of total phenolics (42.13 ± 1.704 tannic acid equivalents mg/g of EEVJ), according to quantitative estimate of phytoconstituent, specifically total flavonoids and total phenolics. The results of the Pharmacognostical standardization of *Vachellia jacquemontii* (Benth.) Ali indicates that stem plays an important role in both conventional medicine and contemporary therapies. Combining phytochemical profiling and morphological evaluations allows for the establishment of thorough standards that maintain traditional knowledge systems while guaranteeing consumer safety and efficacy.

Keywords: *Acacia jacquemontii; Pharmacognostical Standardization; Total Flavonoids and Total Phenolics Content; Antiplatelet; Hypoglycemic*

Abbreviations

mm: Millimetre; EEVJ: Ethanolic Extract of *Vachellia jacquemontii*; QE: Quercetin Equivalent; NaOH: Sodium Hydroxide; $NaNO_2$: Sodium Nitrite; $AlCl_3$: Aluminium Chloride; ml: Milliliter; g/l: Gram/Liter; µg: Microgram; nm: Nanometre; rpm: Rotation Per Minute

Introduction

Vachellia jacquemontii (Benth.) Ali, also called *Jacquemont's acacia* and *Acacia jacquemontii*, belonging to Leguminosae/Mimosaceae/Fabaceae family is indigenous to the Indian and Pakistani Thar Deserts. Certain soil bacteria and this species have a symbiotic connection; the bacteria fix atmospheric nitrogen and create nodules on the roots [1] shrub is also crucial in maintaining the stability of the sand dunes in its natural desert environment [2]. The wood generates a lot of heat, making it valuable as firewood [3]. The strong roots are utilized as rope, while the branches are used to build fences and houses [2]. This herb has been used traditionally for a variety of medical conditions, such as chronic renal disease, snakebite, fever and abortion induction [4].

The plant's bark and leaves have also been used traditionally to treat rheumatism, fever, joint discomfort, and other inflammatory diseases. *Vachellia jacquemontii* (Benth.) Ali's bark and leaves are astringent and contain varying amounts of tannins. When applied topically, the plant's astringent qualities may aid in wound healing and infection prevention. They are applied externally, frequently as a wash, to treat various skin conditions like hemorrhoids, sweaty feet, certain eye issues, and mouthwash, among others [2,3].

Vachellia jacquemontii (Benth.) Ali also produces varying amounts of gum from the stems and trunk. This is occasionally administered internally to treat hemorrhoids and diarrhea [3]. Bioactive substances with antioxidant qualities, such as flavonoids and tannins, are frequently found in acacia plants. These substances might aid in the body's defense against dangerous free radicals, which could help avoid chronic illnesses and promote overall health.

Figure 1: *An overview of plant Vachellia jacquemontii (Benth.) Ali.*

The leaves and bark of *Acacia* species are sometimes used as natural remedies to support digestive health. This includes alleviating indigestion and promoting overall gastrointestinal function. This has been used to reduce fever and as a mild analgesic. This may be related to the anti-inflammatory and analgesic properties of compounds present in plant. Some cultures use *Acacia* plant parts (such as bark or roots) as tonics for general vitality and health. These tonics are believed to help strengthen the immune system and improve overall wellness. Though more research is needed, there are indications that some acacia species might possess hypoglycemic effects, potentially aiding in the regulation of blood sugar levels. Various species of *Acacia* have demonstrated antimicrobial properties against bacteria, fungi, and other pathogens in preliminary studies.

Because of their higher free radical scavenging and anti-oxidant qualities, the diverse genus *Acacia* contains a wide range of bioactive components, including alkaloids, [4] flavonoids, terpenoids, [5] phenolic compounds and tannins [6,7]. These components are responsible for a number of pharmacological and biological properties, including anti-inflammatory, antibacterial, antiplatelet, antihypertensive, hypoglycemic, analgesic, anti-atherosclerotic, and anticancer properties [7].

This suggests that, *Vachellia jacquemontii* (Benth.) Ali have many medicinal potentials although specific research on this species is still scarce. In our present study, our main aim is to conduct a Pharmacognostical standardization of the stem of *Vachellia jacquemontii*. The stem of *Vachellia jacquemontii* is crucial for its rich phytochemical profile, necessitating a thorough evaluation. Morphological/anatomical and histological studies reveal key features like vessel arrangement, fiber types, and cellular structures. Organoleptic evaluations provide insights into sensory attributes influencing therapeutic applications. Pharmacognostical standardization promotes safe use in traditional healing practices and modern therapeutics by combining morphological assessments with phytochemical profiling, ensuring efficacy and safety while preserving traditional knowledge systems.

Materials and Methods

Collection and authentication of the plant material

Stem with pods of *Vachellia jacquemontii (Benth.) Ali* were collected from hills region of Pipaleshwar Mahadev Mandir Budiwada, Barmer (Rajasthan). A voucher specimen has been placed at the Botanical Survey of India (BSI), Jodhpur, Rajasthan, and the plant was identified by Dr. Shriman Lal Meena (Scientist- E and Head of office) vide its voucher number is BSI/AZRC/ 1. 12012/ Tech/2023-24/ (Pl. Id.) /489.

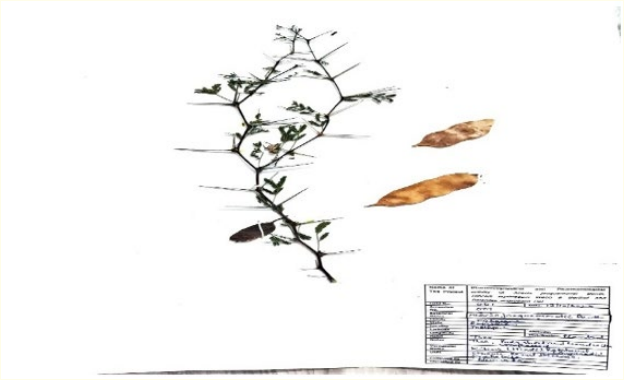

Figure 2: *Herbarium of plant Vachellia jacquemontii (Benth.) Ali.*

Standardization of crude drug

Organoleptic and morphological evaluation

Organoleptic and morphological evaluations include the use of sensory organs to examine the color, odor (smell), taste, size, shape, and texture of crude drugs. As per WHO guidelines, the color, surface characteristics, Odour, fractures, taste, size and shape of stem were evaluated [8].

Microscopic/histological evaluation

Stem was cut into fine section with the help of sharp blade. The finest section was put into safranine solution and allowed to stand for 5 minutes for proper staining. The stained section was put on glass slide with the help of zero size brush and glycerine drop was applied. The section was observed under monocular microscope [8].

Determination of physico-chemical parameters

Determination of total ash value

Ash values are used to identify earthy or sandy materials, low-quality items, and expired medications. Using total ash and acid insoluble ash, they can also be utilized to identify chemical ingredients. Drug powder that has been air-dried is weighed, burnt at 600 degrees Celsius in a muffle furnace till white, and then cooled in a desiccator to calculate the total amount of ash. The residue is wet with water or ammonium nitrate, dried, and burned in a muffle furnace if carbon-free ash cannot be produced [9]. The, % of total ash was calculated using the following formula:

Determination of acid insoluble ash value

45 ml of 1:5 HCl added to a plate, boiled for five minutes, and then filtered to estimate the sample's total ash content. After being collected on ashless filter paper, cleaned with distilled water., the insoluble material moved back to the original dish. After the dish was cooled in desiccators, the filter paper was dried, burned to a constant weight, and the weight was recorded [10]. The formula below was used to determine the percentage of acid-insoluble ash:

Determination of extractive value

Minerals, water, and chemical molecules (1°and 2° metabolites) are all components of plants. Different solvents are used to extract these chemicals in different ratios. The quantity of active ingredients that can be extracted using solvents from specific amount of medicinal plant material is known as its extractive value.

Determination of alcohol soluble extractive value

Weight of air-dried powder drug (Stem's of *Vachellia jacquemontii)* was taken added to 100 ml of 99.99% ethanol, and shaken for 6 hours. After 18 hours, it was filtered and pipette out. The resulting filtered sample was evaporated in a water bath, and then stored in hot air oven at 105°C for 6 hours. After/cooling, the weight was taken [11]. The % of alcohol-soluble extractives value was calculated using the following formula:

Determination of water-soluble extractive value

After being weighed and ground into a powder, the air-dried stem of *Vachellia jacquemontii* mixed with 100 milliliters distilled water and shaken for six hours. It was filtered and pipetted out after eighteen hours. After being filtered, the resultant sample was evaporated in a water bath and then kept for six hours at 105°C in a hot air oven. The weight was taken once it had cooled [12].

The percentage of water-soluble extractive matter was calculated using the formula:

Determination of loss on drying (LOD) at 105°C

Loss on drying refers to weight loss due to water and volatile matter under specific conditions. A 2g of air-dried powder drug of plant's stem was accurately weighed, heated in a hot air oven at 105ºC for 5 hours, and then cooled in desiccator before being weighed again until a constant weight was obtained [13]. LOD calculated using the following formula:

Determination of pH value

A glass electrode and a pH meter are used to measure the pH of an aqueous liquid. A medication that has been roughly ground is mixed with 100 milliliters of water, filtered, and then examined at 25 degrees Celsius. The pH value is expressed as the reciprocal of hydrogen ion concentration [13].

Preparation of ethanolic extract of dried stem of Vachellia Jacquemontii (EEVJ) for phytochemical evaluation

The dried stem of *Vachellia jacquemontii* was powdered, boiled, filtered, and centrifuged to extract a concentrated solution. The supernatant was collected and concentrated, resulting in a one-fourth volume of the its original amount. The solution was auto-claved at 121°C and 15 lbs pressure, and stored at 4°C. for solvent extraction, the powder which was air-dried powder (*Vachellia jacquemontii's* stem) placed in ethanol, plugged with cotton wool, and shaken at 190-220 rpm for 24 hrs. Collection of supernatants and the evaporation of solvent was done to create the final volume, which was stored at 4°C in an air tight bottle.

Qualitative estimation of the phytochemical of the extract

These tested were conducted for the estimation of the presence of proteins, carbohydrates, tannins, alkaloids, glycosides, flavonoids, saponins, and essential oils by using. Standard procedures in extracts [14,15].

Carbohydrates

Molisch's test

- **Procedure**: In extract, add a few drops of Molisch's reagent (α-naphthol solution). Slowly pour strong sulphuric acid along the test tube's walls.
- **Observation**: A red or purple. Ring at interface of the two layers-indicates the presence of carbohydrates.

Benedict's test (for reducing sugars)

- **Procedure**: Heat the plant extract in a boiling water bath after adding Benedict's reagent.
- **Observation**: When reducing sugars shows, the colour shifts from blue to green, yellow, orange, or red.

Proteins

Biuret test

- **Procedure**: Add a few drops of Biuret reagent (a mixture of sodium hydroxide and copper sulfate) to the extract.
- **Observation**: A violet or purple color indicates presence of proteins.

Xanthoproteic test

- **Procedure**: Add concentrated nitric acid to the plant extract and heat.
- **Observation**: Yellow color indicates the presence of proteins (specifically those containing aromatic amino acids).

Alkaloids

Mayer test

- **Procedure**: Add Mayer reagent (potassium mercuric iodide solution) to the plant extract.
- **Observation**: Creamy white precipitate indicates the presence of alkaloids.

Dragendorff test

- **Procedure**: Add Dragendorff reagent (bismuth nitrate and potassium iodide solution) to the extract.
- **Observation**: An orange or red precipitate indicates the presence of alkaloids.

Flavonoids

Shinoda test

- **Procedure**: Few drops of strong HCl are added after a few pieces of magnesium ribbon have been added to the plant extract.
- **Observation**: A pink, red, /orange color indicates the sign of flavonoids.

Alkaline reagent test

- **Procedure**: Add a few drops of sodium hydroxide to extract.
- **Observation**: A yellow- color that turns colorless upon acidification indicates of flavonoids.

Glycosides

Legal test

- **Procedure**: To extract, add sodium nitroprusside solution and then sodium hydroxide.
- **Observation**: A pink, red, or violet colour indicates the of cardiac glycosides.

Keller-Kiliani test (for cardenolide glycosides)

- **Procedure**: Add ferric chloride solution to the extract, followed by glacial acetic acid and sulfuric acid.
- **Observation**: Glycosides are indicated by a brown ring at the contact.

Saponins

Foam test

- **Procedure**: Shake extract with water for a few minutes.
- **Observation**: A prolonged foam that forms is a sign that saponins are present.

Hemolysis test (for saponins in plant extracts)

- **Procedure**: Add extract to a red blood cell suspension and observe for hemolysis.
- **Observation**: If hemolysis occurs, this suggests sign of saponins.

Tannins

Ferric chloride test

- **Procedure**: Add a few drops. of 1% $FeCl_3$ solution to extract.
- **Observation**: A blue, green/blackish colour indicates of tannins.

Lead acetate test

- **Procedure**: Add lead acetate solution to the extract.
- **Observation**: A white precipitate indicates the presence of tannins.

Essential oils

Steam distillation

- **Procedure**: Use a steam distillation setup to extract essential oils from the plant material.
- **Observation**: The distillate will contain essential oils, which can be separated from water.

Odor test

- **Procedure**: Smell the extract directly or after evaporating the solvent.
- **Observation**: A characteristic aromatic smell indicates the presence of essential oils.

Quantitative estimation of phytoconstituents

Total phenolic content

The total phenolic content of extracts was determined using spectrometry [15]. Folin-Ciocalteu's reagent was added to a sample, tannic acid (10 - 100 µg/ml), sodium carbonate (75 g/l), and distilled water. The mixture was stirred for 2 hours at room temperature, and then centrifuged at 2000 rpm for 5 minutes at 760 nm, the absorbance was measured, and a standard curve was created using varying amounts of tannic acid. The findings were given in milligrams of tannic acid equivalents per extract gram.

Total flavonoids content

The total flavonoid concentration of extracts is determined using the colorimetric test with aluminum chloride [16]. A 10 ml volumetric flask filled with distilled water is filled with a quercetin sample or standard solution. 1M NaOH, 10% $AlCl_3$, and 5% $NaNO_2$ are then added. After mixing the solution, absorbance at 510 nm is measured. The results reported as milligrammes of quercetin equivalent (QE)/100 milligrammes of extract.

Results

Organoleptic and morphological evaluation

The organoleptic study revealed that the unpeeled stem of *Vachellia jacquemontii* is cylindrical in shape with rough surface. The peeled stem is yellowish while young stem is reddish in colour. The colour of bark of stem is dark-brown (outer surface) while pale yellow (inner surface). The stem has characteristics odour and astringent and bland taste. The stem is varying in size.

Figure 3: *Stems of plant Vachellia jacquemontii (Benth.) Ali.*

Microscopic/histological evaluation

The transverse section of *Vachellia jacquemontii* dicotyledonous stems having pith (characteristic feature of Dicot stem) layer of xylem and medullary rays (alternate to each other), cambium, phloem, hypodermis, epidermis and trichome (inner to outer side). It also shows the presence of lignified cells having pink colour.

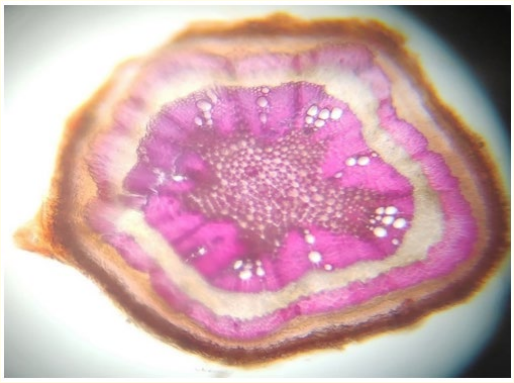

Figure 4: *Transverse section of Vachellia jacquemontii.*

Physico-chemical evaluation of ethanolic extract of dried stem of *Vachellia Jacquemontii*

Any organic material's non-volatile inorganic components make up its ash. An ash residue made up of inorganic materials (silica and metallic salts) is produced when crude pharmaceuticals are burned under controlled conditions. This value is a crucial metric for assessing crude medications because it fluctuates within quite broad bounds [17]. Thus, the proportions of water-soluble, acid-insoluble, and total ash calculated.

The physico-chemical studies of extract, including moisture content, and ash value align with pharmacopoeial standard and previous reports, indicating the drug meets all prescribed parameters and suggests appropriate drug quality (Table 1).

The examination of an extract can also benefits from its extractive value, which provides insight into the types of chemical components present and aids in estimating which compounds are soluble in the given extraction solvent [18]. Results are tabulated in table 1.

S. No.	Standardization Parameters	Results
1	Total Ash content	11.8 ± 0.52% W/W
2	Acid insoluble ash	1.31 ± 0.33% W/W
3	Alcohol Soluble Extractive Value	12.1 ± 0.16% W/W
4	Water Soluble Extractive Value	9.8 ± 0.33% W/W
5	Loss on Drying	3.78 ± 0.18% W/W
6	pH value	6

Table 1: *Physico-chemical evaluation of Vachellia jacquemontii's stem.*

Values in % w/w are expressed as mean ± SEM; n = 3.

Phytochemical evaluation of the ethanolic extract of dried stem of *Vachellia jacquemontii* (EEVJ)

The phyto-chemical screening of the extracts shown the presence of proteins, tannins, alkaloids, flavonoids, amino acids, terpenoids, saponins, and phenolic compounds, while sterols, glycosides, carbohydrates and volatile oils were not present (Table 2).

Compound	Test	Result (Positive/Negative)
Carbohydrates	Molisch's Test	Negative
	Benedict's Test	Negative
Proteins	Biuret Test	Violet/Purple color
	Xanthoproteic Test	Yellow color
Alkaloids	Mayer's Test	Creamy white precipitate
	Dragendorff's Test	Orange/Red precipitate
Terpenoids	Liebermann's Burchard Test	Formation of a blue colour with a mixture of acetic anhydride and concentrated sulfuric acid
Flavonoids	Shinoda Test	Red/Pink/Orange color
	Alkaline Reagent Test	Yellow-to-colorless change
Glycosides	Legal's Test	Negative
	Keller-Kiliani Test	Negative
Saponins	Foam Test	Persistent foam
	Hemolysis Test	Hemolysis of RBCs
Tannins	Ferric Chloride Test	Blue/Green/Blackish color
	Lead Acetate Test	White precipitate
Essential Oils	Steam Distillation	
	Odor Test	

Table 2: *Qualitative estimation of phyto-constituent.*

Quantitative estimation of phytoconstituent

EEVJ found to have a high content of total flavonoids (191.09 ± 1.232 quercetin equivalents mg/g of EEVJ) and a low amount of total phenolic content (42.13 ± 1.704 tannic acid equivalents mg/g of EEVJ), according to the quantitative estimation of phytoconstituent, namely total flavonoids and total phenolics (Table 3).

S. No.	Standardization Parameters	Results
1	Total Flavonoids Content	191.09 ± 1.232
2	Total Phenolic Content	42.13 ± 1.704

Table 3: *Quantitative estimation of phytoconstituent.*

Discussion

Pharmacognostical standardization of medicinal plants is crucial for ensuring their safety, efficacy, and quality. *Vachellia jacquemontii* (Benth.) Ali, of Leguminaceae or Fabaceae or Mimosaceae family is a significant plant in traditional medicine, has garnered attention for its therapeutic potential. This plant is commonly found in several regions of Asia and Africa and is utilized in treating various ailments such as inflammation, infections, and gastrointestinal disorders. The stem of *Vachellia jacquemontii* (Benth.) Ali is crucial for its rich phytochemical profile, which warrants thorough Pharmacognostical evaluation. *Vachellia jacquemontii* (Benth.) Ali is known for various pharmacological and biological properties such as anti-inflammatory, antibacterial, antihypertensive, hypoglycaemic, antiplatelet, anti-atherosclerotic, anticancer, analgesic, free radical scavenging and anti-oxidant properties.

The physiochemical studies of the plant, including ash value and moisture content has been done which was aligned with pharmacopoeial standard and reports, indicating the drug meets all prescribed parameters and suggests appropriate drug quality. Proteins, alkaloids, amino acids, flavonoids, saponins, and other phenolic compounds were found in the plant extracts after phytochemical screening; sterols, glycosides, carbohydrates, and volatile oils were not found. EEVJ had a high content of total flavonoids (191.09 ± 1.232 quercetin equivalents mg/g of EEVJ) and a low content of total phenolics (42.13 ± 1.704 tannic acid equivalents mg/g of EEVJ), according to the quantitative estimate of phytoconstituents, specifically total flavonoids and total phenolics.

The first step in the standardization process involves morphological and histological studies of the stem. Organoleptic evaluations provide insights into the sensory attributes like color, texture, and taste that may influence therapeutic applications. Such foundational knowledge forms the basis for identifying authentic samples versus adulterants or substitutes. Following, phytochemical analysis becomes essential to determine the presence of bioactive compounds within the stem. Establishing a profile of these compounds not only aids in validation but also enhances understanding of their pharmacological mechanisms. Furthermore, this information can be pivotal for regulatory compliance and quality control measures in herbal medicine manufacturing.

Conclusion

In conclusion, the Pharmacognostical standardization of *Vachellia jacquemontii (Benth.) Ali stem plays a vital role in promoting its safe use within traditional healing practices and modern therapeutics alike. By combining morphological assessments with phytochemical profiling, researchers can establish comprehensive standards that ensure both efficacy and safety for consumers while preserving traditional knowledge systems.*

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Conflict of Interest

There is no any financial interest or any conflict of interest.

Bibliography

1. tropical.theferns.info. [Internet]. "Tropical Plants Database". Ken Fern. Checklist dataset (2024).
2. *Vachellia jacquemontii*. Plants of the World Online. Royal Botanic Gardens, Kew (2024).
3. Choudhary K., *et al*. "Ethnobotany of *Acacia jacquemontii* Benth. —An Uncharted Tree of Thar Desert, Rajasthan, India". *Ethnobotanical Leaflets* 13 (2009): 668-678.
4. Clement BA., *et al*. "Toxic amines and alkaloids from *Acacia berlandieri*". *Phytochemistry* 46.2 (1997): 249-254.
5. Haridas V., *et al*. "Avicins: Triterpenoid saponins from *Acacia victoriae* (Bentham) induce apoptosis by mitochondrial perturbation". *Proceedings of the National Academy of Sciences of the United States of America* 98.10 (2001): 5821-5826.
6. Readel K., *et al*. "Tannins from mimosoid legumes of Texas and Mexico". *Economic Botany* 55 (2001): 212-222.
7. Singh R., *et al*. "Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn". *Food and Chemical Toxicology* 45.7 (2007): 1216-1223.

8. Gattuso S., *et al*. "Morpho-histological and Quantitative Parameters in the Characterization of *Lemon verbena* from Argentina". *Boletin Latinoamericano Y Del Caribie de Plantas Medicinales Aromaticos* 7.4 (2008): 190-198.

9. Shilpa P and Upadhyaya A. "Pharmacognostical evaluation of katuka (*Picrorhiza kurroa* royle ex benth". *International Journal of Ayurveda and Pharma Research* 6.7 (2018): 65-69.

10. Shetty SN., *et al*. "A study of standardized extracts of *Picrorhiza kurroa* Royle ex Benth in experimental nonalcoholic fatty liver disease". *Journal of Ayurveda and Integrative Medicine* 1.3 (2010): 203-210.

11. Gami B and Parabia MH. "Pharmacognostic evaluation of bark and seeds of *Mimusops elengi* L". *International Journal of Pharmacy and Pharmaceutical Sciences* 2.4 (2010): 110-113.

12. Thani PR and Sharma YP. "Standardization of Storage Conditions and Duration on Picroside-I and Picroside-II in Raw Material of Drug Kutki (*Picrorhiza kurroa* Royle ex Benth.)". *Nepal Journal of Science and Technology* 17.1 (2016): 23-26.

13. Sharma A and Sisodia SS. "Preliminary phytochemical evaluation of aerial parts of *Sphaeranthus indicus* linn. (asteraceae)". *International Journal of Pharmacognosy* 9.2 (2022): 36-40.

14. Kokate CK., *et al*. "Pharmacognosy". Nirali Prakashan (2005).

15. Khandelwal K. "Practical pharmacognosy". Nirali Prakashan (2006).

16. Singleton VL and Rossi JA. "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents". *American Journal of Enology and Viticulture* 16.3 (1965): 144-158.

17. Mukharjee PK. "Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals". India: Business Horizones (2005).

18. Khandelwal KR. "Practical Pharmacognosy: Techniques and Experiments". Nirali Prakashan (1998).

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