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Original Article

HPTLC, Physicochemical and Phytochemical Analysis of Kodo Millet (*Paspalum scrobiculatum* L.) Seeds with Special Reference to its Ethnopharmacological Review

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ABSTRACT

Background: Kodo millet, botanically (*Paspalum scrobiculatum* L.) is one among the minor millets cultivated mostly in Asian and African continents. Dr. M.S Swaminathan, father of Indian green revolution called all millets as orphan's food. Moreover, most of these millets including Kodo millets have been in use since antiquity by the indigenous communities across the globe for medicinal purpose also. Ayurvedic science also indicates Kodo millet in the management of conditions like diarrhoeal disorders, wound, poison, bleeding disorders, Obesity, Ascitis. In the scenario of escalating significance of millets in light of the United Nations General Assembly's declaration of 2023 as "The International Year of Millets," a scientific analysis of Kodo millet is urgently needed in order to standardize and ensure its quality. **Objectives:** For the purpose of standardizing Kodo Millet seeds, HPTLC, physico-chemical and phytochemical analyses were carried out. Review of literature of its ethnopharmacological uses was also done, because its documentation is essential for creating awareness among the public and scientific community. **Materials and Methods:** HPTLC and physico-chemical analysis of Kodo millet seed were conducted. Alcoholic extraction (reflux) of seed powder was done for phytochemical analysis. The best fingerprint for HPTLC analysis was obtained using Toluene: Ethyl acetate: Methanol: Formic acid as the mobile phase (5:7:3:0.1). Review of literature of its ethnopharmacology was conducted by searching indexed journals and books. **Results and Conclusion:** Physicochemical analysis revealed identity and purity of Kodo millet grains. Proteins, glycosides, tannins and flavonoids were found to be the phytochemicals present in it. The peaks in the HPTLC fingerprint profile correlate to its different phytoconstituents and can be utilized to standardize the drug. Ethnopharmacological review revealed its use in many conditions like Diabetes mellitus, wound healing, bowel cleansing and inflammation. Moreover, it is even used in treating veterinary diseases like Ranikhet disease in poultry and Tympanic disease of cattle.

Keywords: Kodo Millet, HPTLC, Physicochemical, Phytochemical, Standardization, Ethnopharmacology.

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INTRODUCTION

The General Assembly of United Nations declared March 2023 as "The International Year of Millets (IYM 2023)" during its 75th session. Dr. M.S Swaminathan, father of Indian green revolution called all millets as orphan's food. Millets may be grown in arid environments and are resilient to changes in the weather. They are therefore an incredible way for countries to address food scarcity, which will lessen their reliance on imported cereal grains. This year had raised public awareness on nutritional and health benefits of millets. Furthermore, the potential of millets to provide new, sustainable market opportunities for farmers and consumers was also emphasized.[1]

Kodo millet, botanically *Paspalum scrobiculatum* L. is one among the minor millets cultivated in Asian and African continents. Most of these millets including Kodo millets have been in use since antiquity by the indigenous communities across the globe for medicinal purpose also.[2]

Ayurvedic science also indicates Kodo millet in the management of conditions like diarrhoeal disorders, wound, poison, bleeding disorders, Obesity, Ascitis. It is found in several Ayurvedic books under the names Kodrava, Koradoosha, or Udhaalaka. The plant is an annual grass about 100 cm in height, with a short rhizome, glabrous or softly hairy 15-45 cm long leaves, spikelets

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suborbicular glumes. The biconvex, deep purple grains are firmly encased in the stiffened lemma and palea, exhibiting a noticeable ridge along their periphery (Figure 1). As per Ayurveda, the grains are sweet, bitter, astringent in taste, with hot potency. It is constipating, antipoisonous and scrape excess fat, alleviate pitta, kapha humours, aggravates vata humour.[3]

Kodo millet is the most dietary fiber-rich millet available, which makes it an excellent dietary option for diabetics. It contains low fat, protein and certain minerals like calcium, potassium, iron, magnesium and zinc along with vitamins like folic acid, niacin and pyridoxine. Those who are intolerant to gluten or wheat can use it because it doesn't contain gluten. Lecithin, another ingredient in kodo millets, helps to fortify the nervous system. It is beneficial for postmenopausal women with high blood pressure, dyslipidemia, high cholesterol and heart-related conditions and can be used to treat inflammation, hepatopathy, bleeding and overall debility. For treating beri-beri, the stem of the kodo millet plant can be applied topically and the concentrated root juice has diuretic and galactagogue properties.[2] As kodo millet is so easily digested, it can be useful in the formulation of products for both infants and the elderly.[4]

This millet is readily available without a prescription from health food stores or even local marketplaces. Its quality control is therefore extremely important in terms of public health because of the adulteration and substitution issues and hence HPTLC, physicochemical and phytochemical analysis were done.

Kodo millet is not only nutritious but also have medicinal qualities that have been widely used by various indigenous societies to cure various morbidities.[5] Review of literature of its ethnopharmacological uses was also done, because its documentation is essential for creating awareness among the public and scientific community.

MATERIALS AND METHODS

Kodomillet (*Paspalum scrobiculatum* L.) grains, coarsely powdered, were acquired from a nearby Ayurvedic medicine vendor in Poojappura, Pin-695012, Kerala, India. For the purpose of future reference, the voucher specimens (J/Fr/057) were placed at the DravyagunaVijnan museum at the Regional Ayurveda Research Institute in Poojappura, Thiruvananthapuram, Kerala, India. For HPTLC, TLC plates coated with silica gel 60F254 were acquired from Merck in Germany. The other substances, solvents and reagents that were employed were all AR grade.

Physico chemical and Phytochemical analysis

In accordance with the recommended standard procedures, quantitative analyses for loss on drying at 105°C, total ash, acid insoluble ash, water soluble ash, water soluble and alcohol soluble extractive and pH were checked in duplicate.[6,7] The raw material was assessed for physicochemical characteristics and phytochemicals as per the quality standards listed in the Indian Ayurvedic Pharmacopoeia.[8]

HPTLC

Preparation of test samples

CAMAG (Switzerland) HPTLC was used to record the HPTLC finger printing of Kodo millet 0.5 g of the sample is weighed out and refluxed with 10 mL of ethanol for 10 min in a water bath and then filtered. Using a CAMAG ATS4 applicator, 30 and 40 µL each of two tracks were applied on a silica-coated TLC plate (60 F254). It was then developed in a Twin Trough Chamber (CAMAG) measuring 10×10 cm and up to 85 mm from the bottom after being previously saturated with the mobile phase Toluene: Ethyl acetate: Methanol: Formic acid (5:7:3:0.1). Using the CAMAG visualizer, it was photographed in both short UV

Figure 1: Grains of Kodo Millet [*Paspalum scrobiculatum* L.]- at column width.

(254 nm) and long UV (366 nm). To improve the visibility of the bands, the scanned plates were heated to 130°C and derivatized with vanillin sulfuric acid. Photos were recorded and scanned again at λ 575 nm.

Ethnopharmacological review: In this paper, literature search on ethnopharmacological properties was done through search engines, Pubmed and Google Scholarfor locating, selecting and synthesising data.

RESULTS

Physico-chemical Analysis

Physico-chemical studies of Kodo millet- Analyses of loss on drying, pH, volatile oil, water soluble extractive, alcohol soluble extractive, total ash, water soluble ash and acid insoluble ash were carried out as per standard protocol and is tabulated in Table 1.

Phytochemical analysis

Proteins, glycosides, tannins and flavonoids were found in the alcoholic extract of Kodo millet (Table 2).

HPTLC

Paspalum scrobiculatum L. powder was subjected to HPTLC analysis and the results are displayed in Figures 2-4 and Supplementary Figure1 .

The HPTLC analysis sheds light to the presence of many phytochemicals, as shown in the figures. After scanning at UV 254 nm, UV 366 nm and UV 575 nm, the chromatograms (Figures 2-4 and Supplementary Figure 1) were obtained and peak tables were generated. The tables in Figures 3, 4 and Supplementary Figure 1 show the R_f values, peak height, peak area and percent area of the unknown chemicals.

Review: Ethnopharmacological properties

Various tribes use Kodo millet to treat snake bite poisoning, colon cancer, depression, insomnia, stomach disorders, anxiety disorders and migraine. Moreover, owing to the presence of lecithin, Kodo millet leaves are also used to cure stomach problems, joint pain and poisoning due to snakebites.[5]

Baiga and Gond Adivasi indigenous communities of areas like Dindori of Madhya Pradesh are using this as food and medicine to fight growth retardation in children. They have a unique recipe for breakfast called *Kodo patti* bars (Supplementary Figure 2), combining groundnut, soy and kodo. National Family Health

Table 1: Physico-chemical analysis.

Parameters	Results
Loss on drying (%) (at 105°C)	11.34
Total ash (%)	0.62
Acid insoluble ash (%)	0.02
Water soluble ash (%)	0.66
Water soluble extractive (%)	2.03
Alcohol soluble extractive (%)	2.97
pH	6.20
Volatile Oil	Nil

Table 2: Preliminary Phytochemical Analysis.

Tests	*Paspalum scrobiculatum*
Saponins	-
Tannins	+
Phenols	-
Terpenoids	-
Alkaloids	-
Flavanoids	+
Steroids	-
Glycosides	+
Carbohydrates	-
Quinones	-
Proteins	+

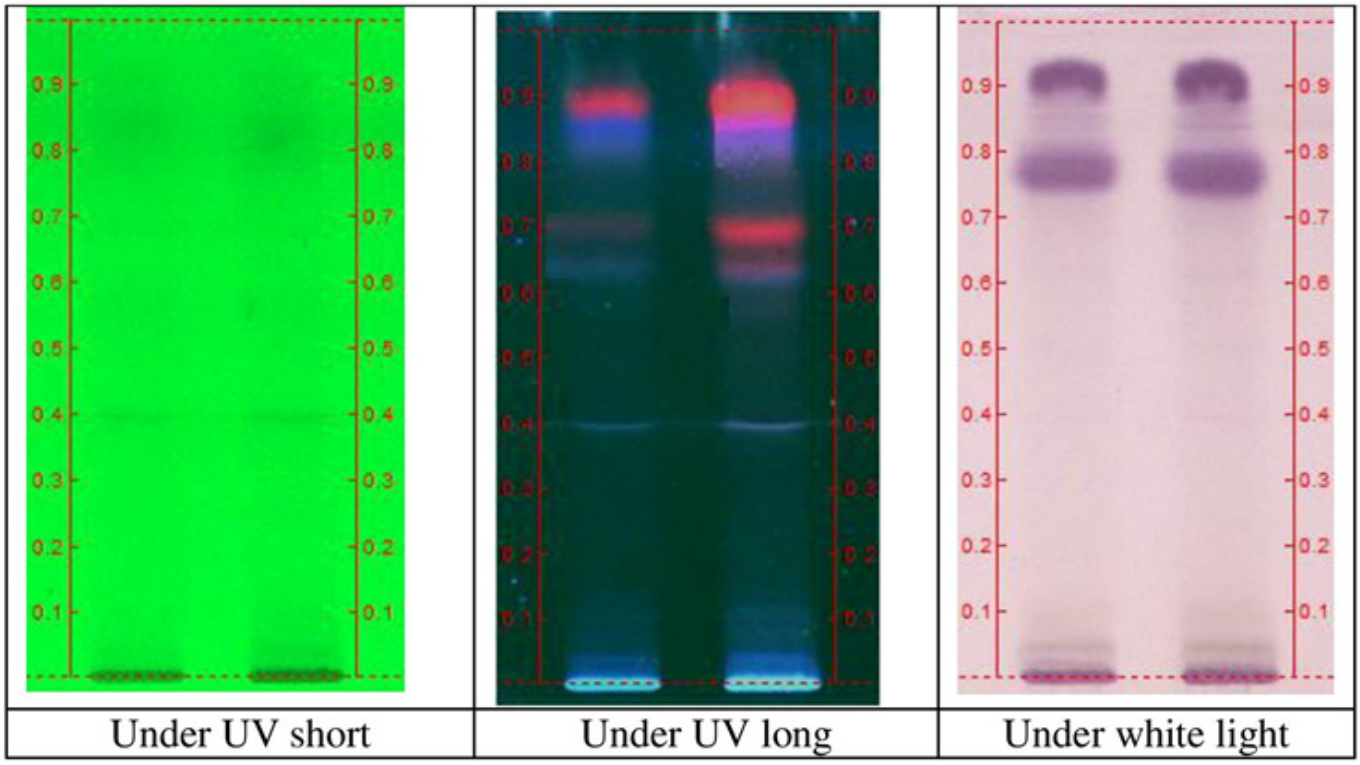


Figure 2: HPTLC fingerprint of *Paspalum scrobiculatum* L. under UV short, UV long and white light after derivatisation- at column width.

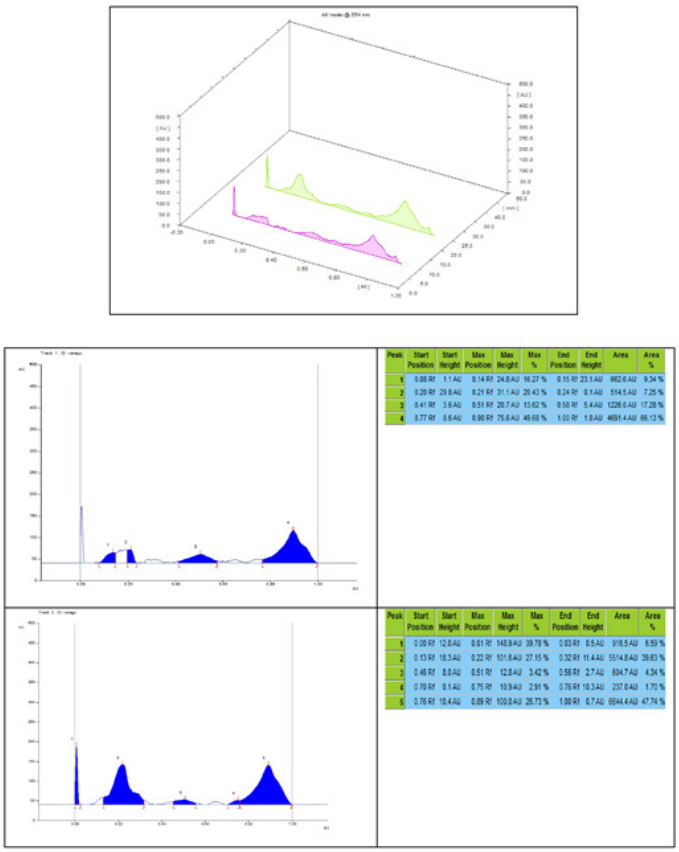


Figure 3: HPTLC chromatographs of *Paspalum scrobiculatum* L. powder at 254 nm- at column width.

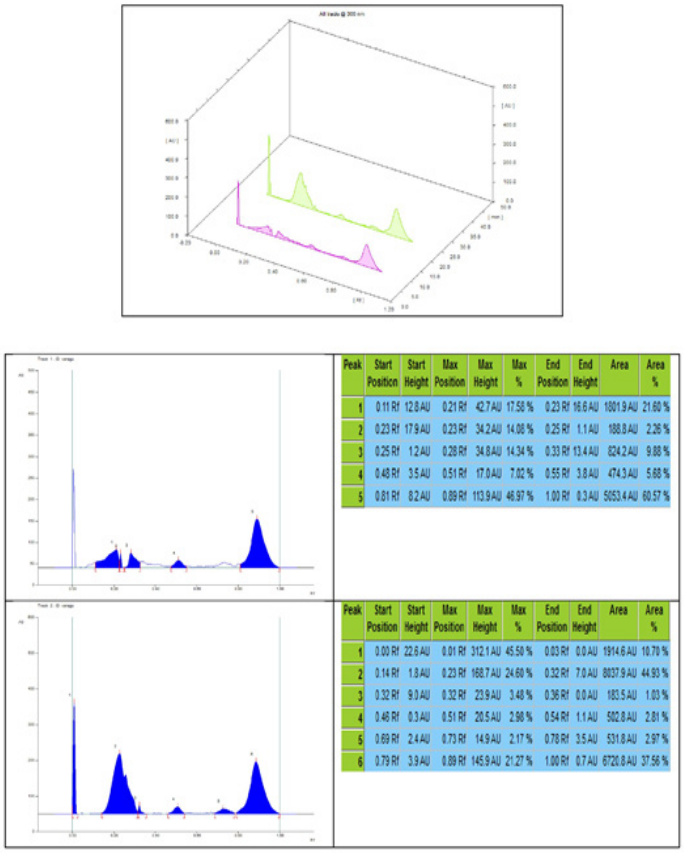
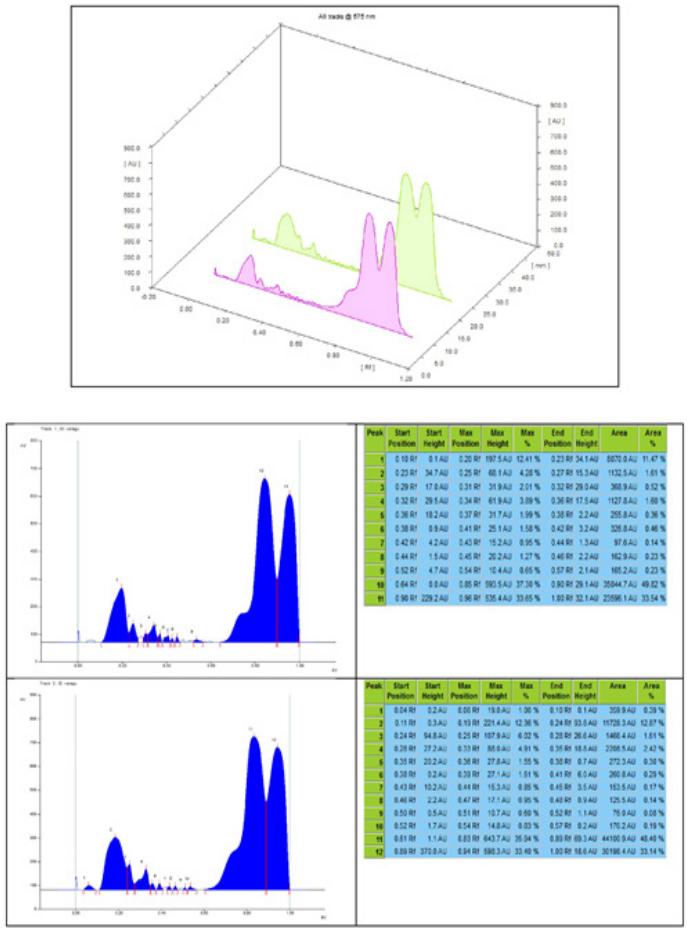


Figure 4: HPTLC chromatographs of *Paspalum scrobiculatum* L. powder at 366 nm- at column width.



Supplementary Figure 1: HPTLC chromatographs of *Paspalum scrobiculatum* L. powder at 575 nm- at column width.

Supplementary Figure 2: Kodopatti bars.

Survey for 2015-16 shows 42% of children aged below five years suffer from stunting and 42.8% are underweight in Madhya Pradesh. The corresponding ratios were 45.8% and 46.6% in the case of Dindori. Due to Iron content, these millets are used by the Gond tribes to tackle iron deficiency anaemia, which is common among tribes especially women.

Tribes in Bastar area of Chhattisgarh utilize Kodo millet grains that are 3-4 years old to treat the highly contagious and deadly "poultry Ranikhet disease" in chicken. Another deadly condition that affects cattle and causes significant stomach enlargement is called tympany disorder, which can be also treated with it. Kodo straw is used in rice fields to prevent leaf rot and blast infections, while grains are combined with bovine feed to increase milk production and save feed costs.

The Kurichya tribes of Wayanad, Kerala, employ the rhizome and root of the kodo millet (*Paspalum scorbiculatum* L.) to treat diabetes and wounds. Kodo millet grains are used to make porridge and mud. The Adiyan tribes of Andhra Pradesh utilizes its root, stem, rhizome and seeds as a vitamin supplement, wound healer, bowel cleanser and for managing diabetes mellitus.[9]

DISCUSSION

For the herbal drug industry, standardization and quality control of botanicals are two crucial obstacles. There could be differences in the nutritional value of medicinal plants and soil composition from batch to batch, which could affect how effective they are. Additional reasons for this fluctuation include seasonal, genetic and natural variability. For the manufacture of herbal medications, standardization is necessary to ensure consistent biological activity, chemical profiling and a quality control process.

The identity and purity of the millet were determined by physicochemical examination. Kodo millet has a moisture level of 11.34%, which indicates that it is somewhat hygroscopic. The physico-chemical examination of Kodo millet grains ruled out the presence of volatile oil and acid-insoluble ash. The total ash provides an estimate of the inorganic content present. It calculates the entire amount of residue that is still present after ignition. It consists of two types of ash: non-physiological ash, which comes from additional minerals and extraneous matter like sand and dirt that sticks to the plant's surface and physiological ash, which comes from plant tissue. It was determined that the overall ash value was 0.62%.

The water-soluble ash value, which represented the water-soluble salts, was determined to be 0.66%. The residue left over after heating the remaining insoluble material to a flame and boiling the entire amount of ash in diluted HCl is known as acid-insoluble ash. This gauges the concentration of silica, particularly in the form of sand and siliceous earth. It was discovered that the acid insoluble ash was 0.02%. Less dissolution was indicated by the estimated water and ethanol soluble extractive values of 2.03% and 2.97%, respectively. The presence of tannins, flavonoids, glycosides and proteins were revealed in the preliminary phytochemical analyses and was further corroborated by HPTLC analysis.

One of the advanced instrumental procedures that take drug quality control to a new level is HPTLC. Chromatographic fingerprinting is an ideal technique for the identification and validation of herbal drugs because a drug's fingerprint remains constant when exposed to the same conditions. The chromatograms for a representative track at 254 nm, 366 nm and 575 nm are displayed in Figures 3 and 4 and supplementary Figure 1. The photo documentation under the UV chamber at 254 nm, 366 nm and after derivatization at 575 nm of the ethanolic extract of the sample was presented in Figure 2. The Kodo millet ethanol extract showed bands with R_f 0.40 under short UV, five bands with R_f 0.6, 0.65, 0.69, 0.85 and 0.90 under long UV and six bands with R_f 0.03, 0.75 and 0.90 under white light after derivatising with vanillin sulphuric acid.

The primary ingredients of Kodo millet, namely proteins, flavonoids, tannins and glycosides are responsible for the observed peaks. The chromatogram and HPTLC images can be counted as authentic finger print of Kodo millet.

Ethnopharmacological knowledge may prove useful in the search and development of new, safe and reasonably priced medications. Its preservation also assists the preservation of culture and tradition of indigenous communities. It is being used in the management of disease conditions like snake bite poisoning, malnutrition, iron deficiency anaemia, Diabetes mellitus, colon cancer, depression, insomnia, stomach disorders, anxiety disorders, wound healing and migraine. Moreover, it is also utilised in various morbidities of animals like Tympani disease of cattle and Ranikhet disease of poultry.

CONCLUSION

The results of this study establish a precise technique that can be used to standardize *Paspalum scrobiculatum* L. grains. The purity of the sample under analysis was confirmed by the physicochemical analysis. Tannins, flavonoids, glycosides and proteins were found in the ethanolic extract according to the preliminary phytochemical analysis and the HPTLC investigation. The current analyses found phytochemicals, which may be the source of its numerous medicinal properties. The ethnopharmacological review showed the utilisation of Kodo millet by various ethnic tribes in numerous human and veterinary morbidities, which underlines the necessity for its promotion and propagation of cultivation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Kodo millet, botanically (*Paspalum scrobiculatum* L.) is one among the minor millets cultivated mostly in Asian and African continents. Dr. M.S Swaminathan, father of Indian green revolution called all millets as orphan's food. Moreover, most of these millets including Kodo millets have been in use since antiquity by the indigenous communities across the globe for medicinal purpose also. Ayurvedic science also indicates Kodo millet in the management of conditions like diarrhoeal disorders, wound, poison, bleeding disorders, Obesity, Ascitis. In the scenario of escalating significance of millets in light of the United Nations General Assembly's declaration of 2023 as "The International Year of Millets," a scientific analysis of Kodo millet is urgently needed in order to standardize and ensure its quality. For the purpose of standardizing Kodo Millet seeds, HPTLC, physico-chemical and phytochemical analyses were carried out. Review of literature of its ethnopharmacological uses was also done, because its documentation is essential for creating awareness among the public and scientific community. HPTLC and physico-chemical analysis of Kodo millet seed were conducted. Alcoholic extraction (reflux) of seed powder was done for phytochemical analysis. The best fingerprint for HPTLC analysis was obtained using Toluene: Ethyl acetate Methanol: Formic acid as the mobile phase (5:7:3:0.1). Review of literature of its ethnopharmacology was conducted by searching indexed journals and books. Physicochemical analysis revealed identity and purity of Kodo millet grains. Proteins, glycosides, tannins and flavonoids were found to be the phytochemicals present in it. The peaks in the HPTLC fingerprint profile correlate to its different phytoconstituents and can be utilized to standardize the drug. Ethnopharmacological review revealed its use in many conditions like Diabetes mellitus, wound healing, bowel cleansing and inflammation. Moreover, it is even used in treating veterinary diseases like Ranikhet disease in poultry and Tympanic disease of cattle

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