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Research Article

Holarrhena Pubescens Wall for Hyperlipidemia

Evaluation of Physico-Phytochemical Parameters of *Holarrhena Pubescens Wall.*Ex G. Don. (Kutaja) Seed and Bark and Their Effect in Obesity Associated Hyperlipidemia

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Abstract: Obesity-related Hyperlipidemia is a major health concern due to its strong association with a number of diseases like diabetes, ischemic heart disease, and renal disease. The safe treatment of Hyperlipidemia thus becomes the need of the hour. This study aimed to evaluate the pharmacognostic, physicochemical, with phytochemical properties of *Holarrhenapubescens* Wall.ex G. Don. *(Kutaja)* seed and bark. The pharmacognostic, physicochemical, and phytochemical evaluation of collected samples of *H. pubescens* seeds and bark was performed and was found to be as per API norms. Furthermore, to evaluate and compare their efficacy in the treatment of Hyperlipidemia. - Forty subjects were randomly allocated to two groups according to the plant part administered to them and was observed for 90 days. The assessment was done on the basis of subjective parameters (Laziness, heaviness of body, Shortness of breath, and sleepiness) and objective parameters (total cholesterol, triglycerides, LDL, HDL, VLDL, and total Cholesterol to HDL ratio) before and after the treatment duration. - Significant decrease in the subjective parameters was recorded, and similarly, a significant decrease in the levels of total cholesterol, LDL, VLDL, and total Cholesterol to HDL ratio was observed for both the seed and the bark of *Kutaja* **–** The therapeutic effect of both the parts i.e. Kutaja seeds and bark were found at par in controlling Hyperlipidemia and thus proved their definite role in ameliorating *Medodusti*i.e. deranged lipid metabolism common in Obesity.– Both seeds and bark can be utilized separately and also in place of one another in cases of Obesity associated Hyperlipidemia

Keywords: Hyperlipidemia; Obesity; *Kutaja* **(***Holarrhenapubescens* Wall. ex G. Don); Cholesterol; Triglyceride; Physico-chemical; Phytochemical analysis.

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1. INTRODUCTION

Hyperlipidemia is a major risk factor in Obesity due to its strong association with cardiovascular diseases (CVD), hypertension, and diabetes-like metabolic disorders.[1] It is recognized as a metabolic disorder owing to elevated levels of total cholesterol, low-density lipoproteins (LDL), triglycerides, and diminution in high-density lipoprotein (HDL).[2] Reports have revealed that 56% and 18% of the patients with high cholesterol levels culminate in ischemic heart disease and hemiplegia, respectively, accounting for 4.4 million deaths annually [1]. It is estimated that CVD may turn out to be the largest cause of mortality and morbidity in India. Recent studies have shown an increase in the number of people affected by diabetes and hypertension in all ages, geographical and socio-demographic areas [3]. Moreover, reports suggest that Indians are more susceptible to high blood pressure, diabetes, and heart attacks by 5-10 years earlier than the western population, mainly in their productive years, thereby causing significant economic losses at individual and national levels [4,5]. In India, the prevalence of diabetes and hypertension is high across all geographical areas in middle and old age and socio-demographic groups. Factors such as the variations in prevalence by state, age group, and rural versus urban are critical to effectively target diabetes and hypertension prevention, screening, and treatment programs for those most in need. With advances in technology and medical science, many potent drugs have antihyperlipidemic activity, of which HMG-COA reductase inhibitors (statins) are the most effective class [6]. Albeit being efficient drugs, statins are also known for a number of side effects they cause such as musculoskeletal conditions like myalgia, myositis, and rhabdomyolysis. The drugs induce an increase in serum transaminase levels, causing hepatic dysfunction. Furthermore, these drugs are reported to be associated with proteinuria and hematuria with the enhanced vulnerability of developing diabetes mellitus [7]. Thus proposing treatment and prevention for Hyperlipidemia induced conditions using a safer and natural alternative becomes a pressing priority. One of the oldest medical systems in the world is Ayurveda which defines life as the union of body, mind, spirit, and senses. Ayurvedic knowledge is based on three main treaties: *Charaka Samhita*, *Sushruta Samhita*, and *Ashtanga Hridaya.* The nature of an individual is constituted by the culmination of the three doshas: *Kapha, Pitta*, and *Vata.* Treatment through Ayurveda focuses on restoring the body-mind matrix using plant-based formulations, which is the aim of *Dravyagunavigyan*i.e. Materiamedica of Ayurved. [8] Ayurveda has immense potential in treating Obesity with Hyperlipidemia as a wide range of easily available, safe, and economical drugs exhibit hypolipidemic activity. Amongst numerous plants known for their therapeutic properties, *Holarrhenapubescens* Wall. Ex G. Don. commonly known as *Kutaja*, is an important plant with significant uses mentioned in classical Ayurveda and many folklore claims. It is a plant with extreme economic importance. The plant's bark has shown significant efficacy in patients with bleeding piles [9]. The plant also has prominent benefits in managing diarrhea and dysentery and is also effective as an antibacterial agent [10]. The hypolipidemic and hypoglycemic activity of the seeds are well documented [11,12] but literature stands insufficient towards revealing the therapeutic activity of *Holarrhena Pubescens Wall.*ex G. Don bark even though both the bark and seeds of the plant have similar components. Thus, the present research was designed to investigate the efficacy of *the Holarrhenapubescens* Wall. ex G. Don Bark on patients suffering from a disorder of fat metabolism (Medodushti) with special reference to Hyperlipidemia and to compare the therapeutic efficacy of *Kutaja* bark with the seed. The present research work was conducted to evaluate the Physico-phytochemical parameters of the *Holarrhenapubescens* Wall.ex G. Don. (Kutaja) Seed and Bark and their effect in Obesity associated Hyperlipidemia.

2. MATERIALS AND METHODS

2.1 Drug collection and authentication

The fresh bark and seeds of *Kutaja* (in Figures 1 and 2) were collected from Ghod bunder in the Thane district of Maharashtra, India, in September, i.e., Sharad Ritu. It was identified and authenticated as *Holarrhenapubescens* Wall.ex G. Don**.** by Head of the Department of Botany Mithibai College, Mumbai (Voucher specimen number - P 07611218)

Fig 1: *H. pubescens* bark

Fig 2: *H. pubescens* seeds

2.2 *Drug standardization and Capsule preparation*

Standardization of *Holarrhena Pubescens Wall*.ex G. Don Bark and the seed was accomplished according to Ayurvedic Pharmacopoeia of India (API).

2.2.1 Pharmacognostic Evaluation

Macroscopic, microscopic, and organoleptic evaluation of the *Holarrhenapubescens* Wall. ex G. Donbark and the seed was performed[13].

2.2.2 Physicochemical evaluation

Foreign matter, pH of 1 % suspension in distilled water, moisture content (loss on drying), ash content, acid insoluble ash, alcohol, and water-soluble extractive value were performed to test physicochemical properties. [13] quantitatively.

1) Foreign matter- The bark and seed were studied for molds, insects, sliminess, rodent fecal matter, and other harmful foreign matter. The process was done by taking 100gms of both samples and spread into a thin layer in a suitable dish or tray. After which, it was observed in daylight with an unaided eye. If any suspected particles are found they are transferred to a petri dish and examined with a 10x lens in daylight.
2) Moisture content (Loss on drying) - This determines the amount of volatile material. For substances containing water, about 10g of the drug was taken, dried in a tarred evaporating dish, dried at 105°C for 5 hours, and weighed. Drying and weighing continued at one-hour intervals until the difference between two successive weighings corresponded to less than 0.25%. Constant weight was reached when two consecutive weighs after drying for 30 minutes and cooling for 30 minutes in a desiccator, showed not more than 0.01 g difference.
3) Total Ash- 2-3gm of the component was weighed and incinerated in a tarred platinum or silica dish at a temperature not exceeding 450°C for 3 hours. After that, the percentage of Ash was calculated and recorded.
4) Acid Insoluble Ash- To the Ash obtained from the above method, 25ml of dilute hydrochloric acid was added. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and weighed without delay. The content of acid-insoluble Ash was then calculated with reference to the air-dried drug.
5) Alcohol Soluble Extractive- 5gm of the air-dried, the coarsely powdered drug was macerated with 100ml of alcohol in a closed flask for 24 hours, frequently shaking for 6 hours and allowing to stand for 18 hours. The solution was filtered rapidly and 25ml of the filtrate was allowed to dry in a tarred flat-bottomed shallow dish to constant weight and measured. The percentage of the alcohol-soluble extract is calculated with reference to the air-dried drug.
6) Water Soluble Extractive- The same process as above was followed by changing the maceration solution from alcohol to water.

2.2.3 Phytochemical Analysis

Qualitative analysis was done to test the presence of flavonoids, phenols, tannins, and saponins. Extracts of *Kutaja*seed and bark were prepared by boiling the powders individually in water for 30 minutes.

a) Test for Tannins (Braymer's test): 0.1 %ferric chloride was added to 2 ml of the prepared extract. Brownish green coloration indicated the presence of tannins. [14]
b) Test for Phenol: 2 ml of 10% lead acetate was added to 2 ml of the prepared extract. White precipitate indicated the presence of phenols. [14]
c) Test for Flavonoids: Few drops of conc. H_2SO_4 was added to 2 ml of the prepared extract. The yellowish-orange color indicated the presence of flavonoids. [14]
d) Test for Saponins: 5 ml of distilled water was added to 2 ml of the prepared extract and shaken vigorously. The formation of lather indicated the presence of saponins[15].

2.2.4 HPTLC analysis

High-Performance Thin Layer Chromatography (HPTLC) was done for active ingredient analysis for both the bark and seed at Guru Nanak Khalsa College, Matunga, Mumbai, India.

2.2.5 Preparation of Capsules

The fine powders of dried *H.Pubescencs* bark and seeds were prepared in the Pharmacy of School of Ayurveda in Navi Mumbai. The bark and seeds were separately powdered with a pulverizer and sifted through mesh sieve no. 80. The hard gelatin capsules were filled with 500 mg of powder in each

capsule and stored in well-covered airtight, clean, and dry containers away from sunlight.

2.3 Study description

It was a comparative randomized clinical trial *Patients selection*: Forty patients suffering from mild to moderate grade hyperlipidemia, between the age group of 18 to 60 years and with BMI between 25 to 35 were recruited irrespective of gender and religion from OPD and IPD of Kayachikitsa Department, Ayurvedic College, and hospital, D. Y. Patil Deemed to be University, Navi Mumbai.

a. *Inclusion Criteria*

- Patients of both genders
- Age between 18 to 60 years
- Patients with BMI between 25-35 kg/m^2 suffering from Mild to moderate-grade Hyperlipidemia
- Patients willing to participate in the trial and abide by to study protocol

b. *Exclusion Criteria*

- Patients suffering from established heart diseases, e.g., HD, CCF, DM, Hypothyroidism, etc.
- Patients taking other Anti-hyperlipidemic drugs like statins
- Patients on drugs which may induce Hyperlipidemia, e.g., Antidepressants, Steroids, Beta-blockers, OC pills, etc.
- Pregnant and lactating women
- Chronic alcoholics

2.4. Ethical Statement

The study was carried out after approval of the Institutional ethics committee (IEC number – DYPU/IEC/2015-16/121). Written consent was received from the participants for the conduction and publishing of the results.

2.5. Treatment

A total of 40 patients were randomly divided into groups A (n=20) and B(n=20). Group A and B were administered 3gm of *H. pubescens* bark powder and seed powder, respectively, before both meals, i.e., 3 capsules twice daily. The study was conducted for 90 days, and follow-up was performed every 30 days till 90 days.

2.6. Assessment

Subjective Parameters: The patients were assessed based on Laziness (*Alasya*), heaviness of body (*Angagaurava*), Shortness of breath (*KshudraShwasa*), and sleepiness (*Nidradhikya*). The patients were graded on a scale from 0-4, with 0 having the least and 4 having the most effect. The examination was done before (0th day) and after the intervention of the drug (90th day).

Objective Parameters: BMI between 25-35 kg/m^{2,}

2.7. Collection of blood samples

Blood samples were collected from the subjects by calling them on the specific follow-up date. In addition, serological laboratory investigations of lipid profile, serum cholesterol, serum triglyceride, HDL, and LDL for all the patients in Group A and Group B were done before (0thday) and after the intervention of the drug - (90th day). Tolerability (Safety) by Global assessment of adverse effects: The tolerability of the drug was tested on the patients and was graded on a scale from 1-4, with 1 indicating excellent tolerability and 4 being poor tolerability leading to the cessation of the treatment.

2.8. Statistical Analysis

Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS v 21.0, IBM). Data on subjective and objective assessment was coded, while lipid data were numerical. For lipid, data for inter-group and intra-group comparison, t-test, and paired t-test were made, respectively. For subjective and objective assessment, the Mann-Whitney U test was used for inter-group comparison, and Wilcoxon Signed rank test was used for intra-group comparison. For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%.

3. RESULTS

3.1. Pharmacognostic Study

3.1.1. Macroscopic and Microscopic Evaluation

Macroscopic and microscopic evaluation are the first steps of establishing the identity and purity of medicinal plants before any further tests are conducted on them. Macroscopic examination characterizes the size, color, taste, and texture, and the microscopic characteristics unfold the histological properties of component [16]. The macroscopic evaluation revealed the outer surface of the *H. pubescens* Wall. The bark is buff to brownish and rough, and wood is sometimes attached to the inner bark. The bark was small re-curved pieces of varying sizes and thicknesses. The bark fracture was found to be short and granular whereas the taste was revealed to be astringent-bitter (*Kashaya-tikta*) On the other hand, the microscopic evaluation showed a wide cortex and secondary phloem with rectangular cork cells in the periderm. In contrast, phelloderm contained prisms of calcium oxalate crystals, unique characteristics of *H. pubescens* bark. The cortex was found to be interspersed with lignified pitted stone cells, and bark exhibited characteristic calcium oxalate with stone cells in parenchyma. Macroscopic evaluation results of the *H. Pubescens* seed showed them elongated with margins curved inside, one side convex and the other concave. The seeds were approximately 1-2cm long with a 0.2-0.3cm thickness. The microscopic evaluation of the seed showed single-layered, radially arranged, compact parenchymatous cells filled with brown content in testa followed by the tegmen that is two layers of small rounded to irregular cells, of which few of them show the presence of prismatic-shaped calcium oxalate crystals. The endosperm was found with outer and inner tangentially elongated epidermal cells covered with cuticles. They are 5-6 layered, rounded to polygonal, compactly arranged parenchymatous cells containing aleurone grains and large oil globules. The seed consists of two foliaceous convolute cotyledons covered with cuticles. Each cotyledon is single-layered tabular epidermal cells towards the dorsal side and rectangular cells towards the ventral side. Our observations were in accordance with the previously published reports on understanding the microscopic and macroscopic structure of *H. pubescens* bark and seeds [17].

3.1.2. Study of Organoleptic Characteristics (Phytosensology)

The study of organoleptic characteristics is a qualitative method wherein a person uses sensory organs to study the characteristic features of crude drugs. This sensory evaluation provides unique information on the significance of the product to provide valid and reliable information to the research department, production, and marketing [18]. For example, organoleptic evaluation of the *H. pubescens* seeds revealed a dark yellowish color with a smooth texture. In contrast, the plant's bark was brown with a rough texture. Both seed and bark have no odor but the seeds had a bitter (*Tikta*) taste and the bark had an astringent-bitter (*Kashaya-tikta*) taste.

3.2. Physicochemical Evaluation

Plants with medicinal properties serve as an important therapeutic aid in ameliorating and preventing human disease, thereby improving the health status of individuals. Investigating the bioactive constituent by estimating the physicochemical and phytochemical properties of the plant reveals its medicinal potential and possible pharmacological effects [19]. Figure 3 represents the physicochemical evaluation of the *Kutaja* bark and seeds. In both the plant material, the foreign matter was not detected and followed the API norm of not exceeding more than 2%. The pH of 1% suspension in distilled water was 5.42% and 5.74% for plant bark and seed, respectively. Total moisture content was calculated by estimating the loss in weight by drying and was revealed to be 8.57% in *Kutaja* bark and 10.42% in seeds. Additional ash content was determined to be 4.6%and 7.05% in *Kutaja* bark and seed, respectively. The ash content constitutes all inorganic elements of the plant, as the ashing procedure eliminates all the organic materials of the sample. This inorganic element comprises minerals such as As, Ca, Fe, Mg, Na, K, Zn, Ni, and Co that function as micronutrients. Furthermore, since ancient times, *SwarnaBhasma* (gold ash) has been used to manifest several diseases and disorders [20] clinically. The acid-insoluble Ash was estimated to be 0.6% in bark and 0.19% in seeds which remains by API norms of not surpassing 1%. The presence of acid-insoluble Ash indicates siliceous impurities and signifies the presence of carbonates, phosphates, oxides, oxalates, and silicates [21]. Extractive values by different solvents assess quality, purity, and the possible presence of adulterating components and are performed to ensure the presence of plant bio-actives and their solubility profile [22]. Alcohol-soluble extractive value was evaluated to be 30.51% and 10.25%, whereas water-soluble extractive value was found to be 33.32% and 27.04% in *H.pubescens*bark and seed, respectively.

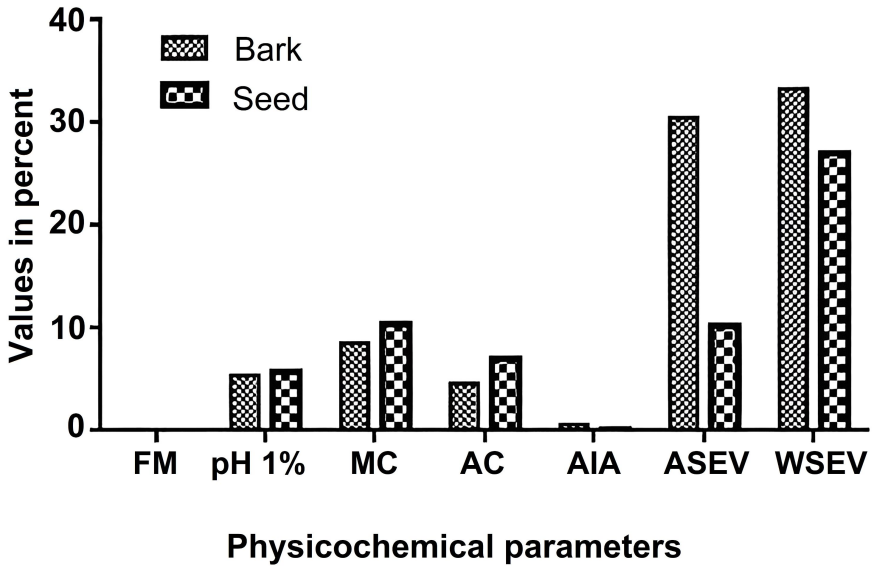

Fig 3: Physicochemical Evaluation of *Kutaja* (*H. pubescens*Wall.ex G. Don.) bark and seed.

FM- Foreign matter; pH 1% - pH 1% suspension in distilled water; MC- Moisture content; AC- Ash content; AIA- Acid insoluble ash, ASEV- Alcohol soluble extractive value; WSEV- Water soluble extractive value.

3.3. Phytochemical Analysis

Phytochemicals are bioactive plant components. These are non-essential nutrients, but their dietary intake promotes health benefits and prevents the body from diseases. Phytochemicals have shown great therapeutic properties alone or when supplemented in combination. For example, tannins have shown anticarcinogenic and antimutagenic properties closely related to their anti-oxidative properties. They also have antimicrobial properties. In addition, reports have suggested that tannin effectively reduces serum lipid levels, modulates immune responses, decreases blood pressure, and accelerates blood clotting.[23]. Phenolic compounds in the diet are vital for antioxidant, anti-aging, anti-inflammatory, anti-inflammatory, and anti-proliferative activities. In addition, it has been shown to decrease the occurrence of diseases like cancer, diabetes, and cardiovascular diseases [24]. Additionally, flavonoids have been reported for their antibacterial, antiviral, antioxidant, and anti-inflammatory properties. They have also been demonstrated to reduce the incidence of many diseases, inhibit cell damage, and be helpful in the DNA repair process [25]. Another phytochemical called saponins possesses antibacterial properties and has been shown to strengthen the immune system by protecting the body against cancer and reducing human cholesterol, glucose, and lipid levels in humans[26]. Phytochemical analysis of *Kutaja* bark and seed has shown the presence of phenols, tannins, flavonoids, and saponins in both the seeds and bark of the plant, as mentioned in table 1indicating its high therapeutic potential.

Table 1: Phytochemical Analysis of *Kutaja* Seed and Bark				
Sr. no	**Test**	***Kutaja* Seed**	***Kutaja* Bark**	**Positive Observation**
1.	Tannins	Present	Present	Brownish Green Discoloration
2.	Phenols	Present	Present	White precipitate
3.	Flavonoids	Present	Present	Yellowish green coloration
4.	Saponins	Present	Present	Lather formation

3.4. HPTLC detection and quantification of conessine

HPTLC is an enhanced technique based on Thin Layer Chromatography (TLC). It is a simple, robust, versatile technique to confirm and quantify the presence of active compounds and identify impurities and adulterating components [27]. Detection and quantification of conessine, a steroidal alkaloid in the aqueous extract of *H. pubescens* bark and seed were determined by HPTLC analysis. HPTLC data of *H. pubescens* bark showed the presence of 4 four prominent peaks (Figure 2). Peak 1 had a max Rf value of 0.04, a max height of 183.9, and a total percent area of 45.44. Peaks 2 and 3 were found a little close to each other with max Rf of 0.43 and 0.55 with a max height of 29.6 and 24.4 respectively. The percent area occupied by peaks 2 and 3 were 13.40 and 23.71, respectively. The last peak of *H. pubescens* bark was observed with total percent area, max Rf and max height as 17.45, 0.82, and 23 respectively. On the other hand, the HPTLC chromatogram of *the H. pubescens* seed revealed two peaks (Figure 2). Peak 1 and 2 occupied a percent area of 41.96 and 58.04, respectively. The max Rf value of peak 1 was 0.04, and peak 2 was 0.83, whereas the max height of peak 1 was found to be 20.1, and that of peak 2 was 28.4.

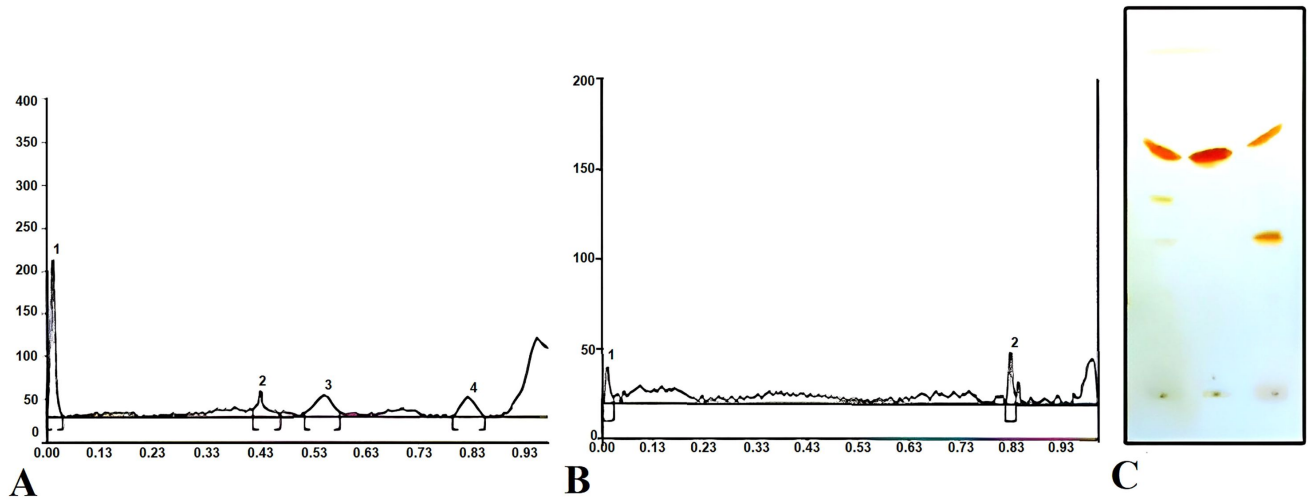

Fig 4: HPTLC Chromatogram of *H. Pubescens*(Kutaja) A. Bark B. Seed

In figure C - HPTLC Fingerprint analysis of 1. Seed extract, 2. Conessine 3. Bark extract
Peak 1 (Seed extract) - Rf value at 0.04 with a max height of 183.9
Peak 2 – (Conessine)- Rf value at 0.43 with a max height of 29.6
Peak 3 (Bark extract) - Rf value at 0.55 with a max height of 24.4

Table 2: HPTLC Analysis of *Kutaja*Bark and Seed										
	Peak	**Start Rf**	**Start Height**	**Max Rf**	**Max Height**	**Max%**	**End Rf**	**End Height**	**Area**	**Area%**
***Kutaja*Bark**	1	0.03	7.3	0.04	183.9	70.49	0.06	1.7	1379.7	45.44
	2	0.41	7.8	0.43	29.6	11.34	0.47	3.0	406.8	13.40
	3	0.51	4.6	0.55	24.4	9.37	0.58	5.4	719.8	23.71
	4	0.79	1.3	0.82	23.0	8.80	0.85	0.1	529.8	17.45
***Kutaja*seed**	1	0.03	0.1	0.04	20.1	41.45	0.05	3.7	143.8	41.96
	2	0.82	0.7	0.83	28.4	58.55	0.84	4.1	198.9	58.04

4. DISCUSSION

The study was carried out after approval of the Institutional ethics committee (IEC).

4.1. Demographic Analysis

Demographic data of a population comprises an array of information that helps generalize the population by giving an aggregate picture(French, 2014). A demographic analysis of forty study subjects in both groups revealed the subjects under study had no significant difference concerning age, gender, diet, and habit, and the population was divided uniformly. In our study, all the patients were randomized into two groups, i.e., A and B. Based on age, 1 (2.5%) patient was in the age group of 18-30 years, 6 (15%) patients were in the age group of 31-40 years, and for the age group 41-50, there were 20 (50%) patients and 13 (32.5%) patients for the age group 51-60 years. It has been observed that subjects in the age bracket of post 41-50 are more vulnerable to the manifestation of Hyperlipidemia. Improper diet, sedentary and unhealthy lifestyle, stress, and addictions are the leading etiological

factors in this age group [28]. The study population was divided by gender, and we recorded the participation of 23 (57.5%) males and 17 (42.5%) females in the present study. The distribution was not statistically significant, reflecting the randomness and unbiased nature of the study population. Socioeconomic wise distribution of the subjects revealed that 5 (12.5%) patients were in the lower class, 11 (27.5%) were in the lower-middle class, whereas 19 (47.5%) in the upper-middle class and 5 (12.5%) belonged to the upper class. Socio-economic status does influence the susceptibility of Hyperlipidemia. Previous reports have suggested that stress, fatty food, and a sedentary lifestyle are predisposing factors for hyperlipidemia [29]. A maximum number of study subjects were job holders. Many epidemiological studies showed that excessive sitting time in the office and a sedentary occupation correlated positively with hyperlipidemia [30]. Diet is known to influence an individual's health. 9 (22.5%) subjects involved in our study were vegetarian, while the remaining 31 (77.5%) had mixed diets. In a mixed diet, non-vegetarian food items like eggs, chicken, mutton, and seafood are the causative factors for Hyperlipidemia. One of the possible reasons for Hyperlipidemia is overnutrition. Thus people indulging in mixed diets are more prone to acquire hyperlipidemia [31].

4.2. Clinical Assessment

The participants were assessed before and after the study for gradation in subjective parameters such as Laziness (*Alasya*), heaviness in the body (*Angagaurava*), Shortness of breath (*KshudraShwasa*) and sleepiness(*Nidradhikya*). In addition, serological laboratory investigations of lipid profile tests such as serum cholesterol, serum triglyceride, HDL, and LDL were performed for every patient of groups A and B on the 0th day and after the 90th day, i.e., before and after the intervention.

4.3. Subjective parameters

Alaska or Laziness is the state of physical inactivity that may further lead to diseases such as type 2 diabetes, coronary heart disease, obesity, dementia, mental illness, and some cancers [32]. In group A, 29 subjects reported Laziness before therapy which got reduced to 23 post-consumptions of 3gms of *Kutaja* bark powder in capsule form orally for 90 days (Group A), marking 20.60% relief from the symptoms (Table 3). Likewise, in group B, 28.13% relief was noted after oral intake of *Kutaja* seed powder. The Alasya recorded before and after treatment was statistically significant in both groups (Group A: p-value = 0.014 and group B: p-value = 0.003) (Table 4). A majority of subjects (33 in group A and 32 in group B) had the complaint of *Angagaurava*which was relieved to 41.38 and 37.50% in group A and B respectively after intervention (Table 3). Intragroup comparison of *Angagaurava*symptoms in both the groups was found to be extremely statistically significant ($p<0.001$), indicating the potential of *Kutaja* bark and seed powder to ameliorate these subjective symptoms (Table 4). Heaviness in the body indicates deviation from an individual's normal well-being; hence, reducing this symptom points out the normalization of the health status. Shortness of breath is the sensation of uncomfortable breathing and can have varying degrees of intensity. Depending on cause and intensity it should be treated otherwise the patient may develop respiratory failure that may prove to be fatal [33]. In our study, 24.14% of subjects were relieved of *Kshudra-Shwasa*in Group A whereas 28.57% relief was observed in Group B. Excessive sleepiness has shown disruption of social and occupational functions and impairs day-to-day activities [34]. In context to *Nidradikhya*, 34.48% relief was observed in Group A whereas 43.33% relief was observed in Group B (Table 3). Further the intragroup comparison of *Kshudra-Shwasa* and *Nidradikhya*un unfolded the fact that after therapy the symptoms were relieved significantly directly reflecting the symptoms mitigating nature of *Kutaja* (Table 4). Inter-comparison of the group's scores was not found to be statistically different from each other. In our study, the symptom of Laziness was cured maximally up to 43.3% in group B (seed powder). A detailed insight into the patient's response gradation wise is provided in Table 6 for both the groups in association with all the four subjective symptoms. 14 subjects from group A and 11 from group B did not show any improvement in Laziness, but a mild improvement in the rest of the subjects was observed. Heaviness in the body showed mild improvement in groups A and B by 60%, and the remaining subjects showed no improvement. In Group B, 8 subjects showed mild improvement in Shortness of breath whereas 7 in Group A. Only 1 subject from Group B for Sleepiness exhibited moderate improvement for all subjective parameters. 11 subjects from group B and 50% from Group A showcased mild improvement. The rest of the subjects did not show any response. No good response was observed for any subjective parameter for both group. Upon comparison of overall subjective parameters, both *Kutaja* bark and seed powder, when given before meal for 90 days in a dose of 3gms, were promising in relieving the Hyperlipidemia associated symptoms supporting previous studies that had reduced symptoms associated with hyperlipidemia [35].

Table 3: Percentage of Relief in Symptoms before and after therapy

Sr. No.	Symptom	Relief in Symptoms (%)					
		Group A			Group B		
		BT	AT	% Relief	BT	AT	% Relief
1	*Alasya*(Fatigue)	29	23	**20.69%**	32	23	**28.13%**
2	*Angagaurava* (Heaviness in the body)	33	21	**41.38%**	32	20	**37.50%**
3	*KshudraShwasa*(Shortness of Breath)	28	21	**24.14%**	28	20	**28.57%**
4	*Nidradhikya*(Exess sleep)	29	19	**34.48%**	30	17	**43.33%**

Table 4: Intra-group (Within the Group) comparisons of Group A and B for Subjective Parameters

Sy Symptom	Group	Sub-group	Mean ±SD	p-value
Alasya(Fatigue)	Group A	BT	1.45 ± 0.76	0.014*
		AT	1.15 ± 0.81	
	Group B	BT	1.60±0.82	0.003**

		AT	1.15±0.67	
Angagaurava (Heaviness in the body)	Group A	BT	1.65 ± 0.88	0.001**
		AT	1.05 ± 0.76	
	Group B	BT	1.60±0.82	0.001**
		AT	1.00±0.72	
KshudraShwasa (Shortness of Breath)	Group A	BT	1.40 ± 0.82	0.008**
		AT	1.05 ± 0.76	
	Group B	BT	1.40±0.82	0.005**
		AT	1.0±0.65	
Nidradhikya (Excess sleep)	Group A	BT	1.45 ± 0.94	0.002**
		AT	0.95 ± 0.89	
	Group B	BT	1.50±1.0	0.001**
		AT	0.85±0.75	

Table 5: Assessment of Subjective parameters in both the groups post therapy

Subjective Parameters	Responses with gradation	Group A: % Subject (N)	Group B: % Subject(N)
Fatigue	Unchanged (0)	70% (14)	55% (11)
(*Alaska*)	Mild (1)	30% (6)	45% (09)
	Moderate (2)	0	0
	Good (3)	0	0
Heaviness of body	Unchanged (0)	40% (8)	40% (8)
(*Angagaurava*)	Mild (1)	60% (12)	60% (12)
	Moderate (2)	0	0
	Good (3)	0	0
Shortness of Breath	Unchanged (0)	65% (13)	60% (12)
(*Kshudra-Shwasa*)	Mild (1)	35% (7)	40% (8)
	Moderate (2)	0	0
	Good (3)	0	0
Sleepiness	Unchanged (0)	50% (10)	40% (8)
(*Nidradhikya*)	Mild (1)	50% (10)	55% (11)
	Moderate (2)	0	5% (1)
	Good (3)	0	0

4.4. Objective parameters

Class I Obesity BMI of 30-34.9 kg/m^{2}dyslipedemia was observed. Hyperlipidemic condition is a situation wherein there is a rise in serum levels of triglycerides (>150mg/dL) and/or total cholesterol (>200mg/dL) [36]. To investigate the repercussions of *Kutaja*bark and seed powder on objective parameters of Hyperlipidemia, a complete lipid profile of all the patients was estimated, encompassing levels of serum cholesterol, triglycerides, HDL, LDL, and VLDL. In Group A, the mean level of serum total cholesterol was 242.35 mg/dl before treatment which got reduced to 209.70 mg/dl after 90 days of treatment likewise in Group B mean cholesterol level was reduced from 239.35 mg/dl to 211.65 mg/dl. Similarly, serum triglyceride levels were normalized from 178.45 mg/dl to 164.22 mg/dl for groups A and B, 176.15 mg/dl to 164.67 mg/dl. Extremely significant mitigation was seen in the total cholesterol (p >0.001; Table 6). Group B showed a significant (p=0.093) reversal in the levels of triglyceride post-therapy, albeit non-significant changes were recorded in group A (Table 6) both LDL and HDL harbor lipid and protein components. HDL is considered a good lipoprotein as it transports cholesterol to the liver for excretion in the bile. In contrast, LDL transports it to all tissues other than the liver; therefore is considered a bad lipoprotein [37]. In Group A, mean levels of LDL reduced from 161.88 to 130.19mg/dL and 167.35 to 134.66mg/dL in group B after 90 days of treatment. Mean levels of HDL increased from 40.16mg/dL and 40.44mg/dL to 47.31mg/dL and 47.88mg/dL in groups A and B, respectively. Upon intragroup comparison, changes in levels of LDL and HDL were found to be highly significant for both groups (Table 7). VLDL is formed when excess fatty acids are converted to triacylglycerols and cholesterol to cholesteryl esters. Removal of triacylglycerol further forms LDLs [38-41]. In group A, the mean level of VLDL was 35.63 mg/dl before treatment, which got reduced to 24.69 mg/dl; in group B it reduced from 36.83 mg/dl to 26.04 mg/dl. A higher ratio of serum total cholesterol to HDL marks a higher risk of heart disease. In both groups, this ratio decreased after therapy and was recorded to be statistically significant upon intra-group comparison (p<0.001). Inter-comparison of the group scores was not found to be statistically different from each other. Thus, from the above data, we can infer that consumption of *Kutaja*seed or bark can significantly ameliorate Hyperlipidemia-induced changes in the lipid profile of the patients. Also, there was no significant difference between the effectiveness of seed and bark and both were revealed to be the potential in relieving objective parameters of hyperlipidemia.

Table 6- Intragroup (Within the group) comparison of Group A and B for Objective Parameters

Parameters	Group	Pairs	Mean ±SD	p-value of paired t-test
Total Cholesterol	Group A	BT	242.35±34.70	0.000**
		AT	209.70±36.18	
	Group B	BT	239.35±28.911	0.000**
		AT	211.65±29.35	
Triglycerides	Group A	BT	178.45±77.198	0.120#
		AT	164.22±58.66	
	Group B	BT	176.15±65.142	0.093#
		AT	164.67±56.38	
LDL	Group A	BT	161.88±23.81	0.000**
		AT	130.19±26.97	
	Group B	BT	167.35±21.29	0.000**
		AT	134.66±25.53	
HDL	Group A	BT	40.16±7.37	0.000**
		AT	47.31±9.32	
	Group B	BT	40.44±6.87	0.000**
		AT	47.88±8.81	
VLDL	Group A	BT	35.63±9.61	0.000**
		AT	24.69±7.16	
	Group B	BT	36.83±9.68	0.000**
		AT	26.04±8.65	
Total Cholesterol / HDL ratio	Group A	BT	6.25±1.57	0.000**
		C AT	4.61±1.29	
	Group B	BT	6.06±1.10	0.000**
		C AT	4.56±1.06	

*** Highly Significant, # Not significant, n = 20 in each group*

4.5. Evaluation of Tolerability (Safety) by Global assessment of adverse effects

Not a single adverse event was reported in any of the study subjects during or after the study. It showed that there was excellent tolerability of interventions in both groups.

5. CONCLUSION

The pharmacognostic, physicochemical, and phytochemical evaluation of collected samples of *H. pubescens* seeds and bark was performed and was found to be as per API norms. *H. pubescens*is a medicinal plant known to have anti-hyperlipidemic activities. Patients, when treated with *H. pubescens* seed and bark powder separately, showed a marked amelioration in subjective parameters like fatigue (*Alasya)*, heaviness of body *(Angagaurava*,) shortness of breath *(Kshudra-Shwasa)* and Sleepiness *(Nidradhikya)* and the results were found to be statistically significant post-therapy. Administration of *Kutaja* seed or bark can bring about significant amelioration in Hyperlipidemia induced changes in the lipid profile of the patients, as inferred from the results. Kutaja, because of its bitter and pungent component, has *medohar* effect. The present study projects at par efficacy of *Kutaja*bark and seed powder in treating hyperlipidemia cases in clinical settings. The present study also lays the foundation for extensive research on *Kutaja* bark and seed powder, considering more population and longer duration to unfold the long-lasting effects of this medicinal plant.

6. AUTHORS CONTRIBUTION STATEMENT

Rajkumar Gupta and Shradha Nayak conceptualized and designed the study, and Ombeer pal and Mohan Yende curated the data and prepared the original draft. Ritu Wadhwa, Arundhati Banerjee, and Kanchanlata Tungare discussed the methodology and analyzed the data. MustansirBhori, Shishira Bharad wajand Dnyaneshwar Padvi Provided valuable inputs towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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