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Harvesting, phytochemical analysis and medicinal importance of *Holarrhena antidysenterica* (L.)

Sneha Singh and Neeta Singh

Abstract
Holarrhena antidysenterica R. Br. Sans (Kutaj) belonging to family Apocyanaceae is a small tree or shrub. Its bark is used as an astringent, anthelmintic, stomachic, febrifuge, diuretic, and is useful for piles, dyspepsia, asthma, amoebic dysentery, and other stomach ailments. Increased demand and the harmful harvest of bark have led to the decline of this important tree. Research is being done to establish sustainable harvesting methods for stem bark and the suitability of other plant parts. Various methods were used to harvest the bark. In these methods the tree trunk was divided into three or four equal parts and the bark was removed from one part and the harvesting was done by removing the strands of length from the main trunk of the tree. Other fibers / resistors were also tested in small / small trees. Samples of harvested bark and parts of various plants such as branch bark, wood, flowers and leaves are analyzed to obtain tannins, phenols, total alkaloids and complete flavonoids. Phytochemical analysis revealed that the active ingredients in trunk bark were comparatively higher, i.e., total phenols (7.51±0.12%), total flavonoids (0.19±0.09%), total alkaloids (2.25±0.06%), and tannins (8.61±0.10%) than other plant parts studied. Strip harvesting was found to be the best method for harvesting and by this method the bark can be harvested on sustainable basis after every 18 months. Bark should be harvested by removing only outer and middle bark leaving the inner bark for regeneration.

Keywords: Alternative plant parts, bark harvesting, Holarrhena antidysenterica

Introduction
Medicinal plants, since ancient times, have been used in almost every culture as a source of medicine. About a third of the world's population relies on plants and plants extracted from their health care. India, represented by rich culture, traditions, and diversity, offers a unique opportunity for researchers (Jachak and Saklani, 2007) [1]. India resides in a gold mine of well-documented and well-executed knowledge of traditional medicine. The last decade has seen a resurgence of interest in and use of medicinal plants and medicinal products. Herbal medicines are still the backbone of about 75-80% of the world's population, especially in developing countries, in primary health care due to better cultural acceptance, better adherence to the human body and less side effects. In recent years, however, there has been a dramatic increase in their use in developed countries. Forest areas have been a source of medicinal plants and herbs for centuries. This position cannot continue because on the one hand the forest floor has been declining slightly and on the other hand the demand for medicinal plants and herbs is growing exponentially. This has led to scientific controversy over the exploitation of medicinal plants in forests. This problem is also exacerbated by crop failure and uncontrolled crop damage, which is detrimental to the health of millions of people who rely on medicinal plants.
The bark of the tree is the most widely used traditional tree in the world (Cunningham and Mbenkum, 1993) [2]. The growing demand for medicinal bark, for sale, and for destructive harvesting methods is a major threat to the highly sought-after forest species. Also, a large portion of rural communities in particular still use traditional medicine, which contains the most important herbs harvested from natural forests. Given the importance of the medicinal plant industry and the dependence of communities on traditional medicine, the uncontrolled, destructive harvesting of tree bark (Mander, 1995, Grace, 2002 and Grace, *et al.* 2002) [3-5]. In the natural forest is a growing concern. Good collection practices are needed for the longevity of wild people and their habitats. Medicinal plant materials need to be collected in a timely manner to ensure the best quality for both the first and the finished product (WHO, 2003) [6]. It is necessary that the harvesting methods used should not be harmful. In India, the demand of *Holarrhena antidysenterica* is 1000-2000 MT annually (Ved and Goraya, 2007) [7].

Excessive bark harvest affects availability of *H. antidysenterica* (Kutaj) population in the forest areas of central India. The species has decreased alarmingly due to illegal logging and unsustainable harvest of bark. Keeping the view in to consideration a study was conducted for sustainable harvesting of *H. antidysentrica* (Kutaj) bark in Tropical Forest Research Institute, Jabalpur, M.P.

H. antidysenterica (commonly known as Kutaj) belonging to family Apocyanaceae, is a small tree or shrub. It is found in Asia, Africa, Madagascar, India, and Philippines. This tree grows throughout India up to an altitude of 4,000 ft. and often gregariously found in deciduous forests, open waste lands and is especially abundant in the sub-Himalaya tract (The Wealth of India, 1997) [8]. *H. antidysenterica* is up to 13 m in height, with milky latex, its bark peels off in flakes and is grey to pale brown in color. The leaves are shiny on the upper surface, dull and hairy on the lower, opposite, subsessile and elliptic. The flowers are white, in terminal corymbose cymes; the fruits are cylindrical, dark grey with white specks and occur in pairs; the seeds are light brown and 0.5-1.5 cm in size. Around 30 alkaloids have been isolated from the plant, mostly from the bark. These include Conessine, Kurchine, Kurchicine, holarrhimine, conarrhimine, conaine, conessimine, iso-conessimine, conimine, holacetin, and conkurchin (Kumar *et al.* 2007) [9].

Kutaj leaves, bark, and fruit are useful for various ailments. However, bark is a very useful component and is used as an astringent, anthelmintic, antidontalgic, stomachic, febrifuge, antidropsical, diuretic, in piles, colic, dyspepsia, asthma, and as a remedy for skin and spleen diseases. A hot decoction of the drug is used as a gargle for toothache (Akhtar *et al.* 2011) [10]. A well-known remedy for amoebic dysentery and other stomach ailments (CIMAP, 1992) [11]. To this end the bark is harvested by cutting down the entire tree and cutting off the main trunk and branches and removing all the bark from the existing tree. Bark scrutiny has caused a great deal of damage to wildlife, including forest trees.

Destructive Harvesting Practices: Demand for Kutaj bark is increasing worldwide, which has led to a reduction in this important resource for medicinal plants. Current methods of harvesting medicinal plants from forests involve particularly harmful processes. Harmful methods of harvesting include stripping the tree completely to get its bark or cut it down to make it easier to harvest. Medicinal plants in which the bark is useful part of it is in great danger as the bark of the trees is removed by heat from the trunk of the tree. The only possible way to meet this growing need is to harvest the bark in such a way that it should not interfere with the health and growth of the trees. It is therefore desirable to measure sustainable harvesting methods with scientific experiments.

Sustainable Harvesting Practices: Sustainable use has been defined as the use of biological components in a way that does not lead to a long-term decline in biodiversity, thus maintaining its ability to meet the needs and aspirations of present and future generations.

Sustainable harvesting is possible with protection and various methods. In general, fire and veld protection, enhancing youth regeneration, controlling extraction, informing the use of medicinal plants as specific steps for the continued harvesting of medicinal plants. Sustainable bark harvesting systems rely heavily on the response of target species to bark removal. The amount of bark that can be harvested under different harvesting orders will depend largely on livestock growth and target species growth, bark characteristics (especially bark thickness), and the rate of re-growth of post-harvest bark. Different types of forests and forests react differently to bark removal, both in terms of wound closure and exposure to pests and fungi. Therefore bark harvesting programs for therapeutic purposes must be accompanied by specific species. Bark harvesting to ensure the availability of sustainable treatment bark is the only way to harvest those species that recover after the removal of the bark by the development of sheets or edges. Key features of the bark harvesting system include width and height, crop rotation, minimum size of harvested trees, percentage of trees that will be exposed to bark removal, and number and rotation of leaflets in selected trees.

Materials and Methods

Study Areas

Study areas were selected in the forest of Ghunghuti Distt. Umariya (M.P.). Surveys were conducted in different forest areas of the states to select Kutaj growing areas. Populations of selected species were identified with the help of local people and forest officials. Experiments were laid out for standardization of sustainable harvesting of species in different forest areas. Randomized design with three replications was used to lay out the experiments. Trees of different age group and girth size were selected for laying out the experiments. Care was taken not to include trees with pollarded crown, broken branches, or those infected with fungi and insects.

Methodology

Bark harvesting

Three methods of bark harvesting were studied.

- Method I: Tree girth was divided into four equal parts and the bark was extracted from one part.
- Method II: Tree girth was divided into three equal parts and the bark was extracted from one part.
- Method III: Strip bark harvesting conducted by removing longitudinal alternate/opposite strip on the main trunk of the tree.

Chemical Analysis: The harvested bark samples and plant parts were brought to the laboratory for chemical analysis. The harvested samples were dried under shade. The fresh and dry weights of the bark were recorded. The dried bark samples were ground into coarse powder and used for chemical analysis. Bark and other plant parts were quantified for the estimation of active chemical ingredients, i.e., total phenols, total alkaloids, tannins, and total flavonoids content. Total phenols in the samples were estimated by Folin-Ciocalteau method (McDonald *et al.* 2001) [12], total alkaloids by Magnesium Oxide method (Sahu, 1983) [13], tannins by Folin Denis method (Schanderi, 1970) [14] and for estimation of total flavonoids, aluminum chloride colorimetric technique was used (Chang *et al.* 2002) [15].

Statistical Analysis: Data on recovery and quality characteristics of Kutaj bark were analyzed statistically using multivariate ANOVA and variation in the quality characteristics of different plant parts were tested by one way ANOVA using (Statistical Package for the Social Sciences (SPSS), Version 14.0) and values of $P \leq 0.05$ were taken to imply statistical significance. Statistically best harvesting method and season were determined using Duncan's Multiple Range Test (DMRT) by SPSS. Means were calculated from

seven replications and results were expressed as the mean±SD.

Observations

Data on regrowth (regeneration of bark) was recorded half yearly. The bark's regenerative properties were determined by the time taken to regenerate the bark. The stage of bark recovery varied from tree to tree. The physical appearance of bark regrowth was recorded. Two types of bark regrowth were observed, i.e., edge growth and sheet growth. Insect and fungal attack incidences were also recorded.

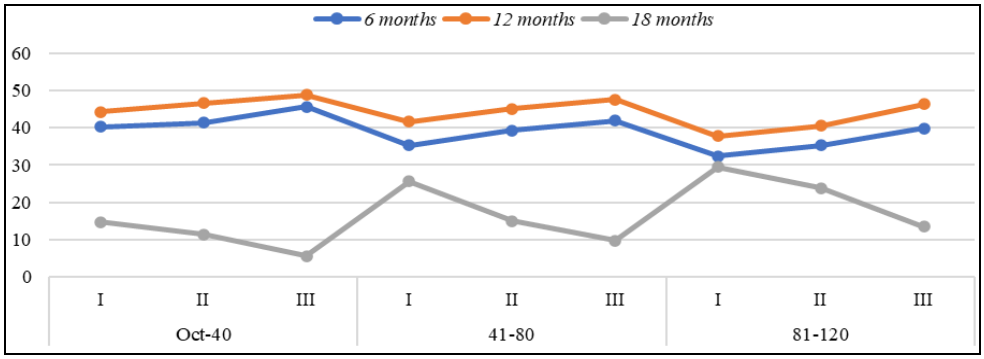
Results and Discussion

Bark regrowth was represented as regeneration percentage observed at six months intervals. In girth at breast height (GBH) group 10−40 cm bark regeneration percentage was faster in method III, i.e., strip harvesting (45.62±0.49%) followed by method II (41.35±0.36%) during initial 6 months period. Similar trends were observed during consecutive period and complete regeneration was achieved within 18 months after harvest. With respect to 41−80 cm and 81−120 cm GBH group similar bark regeneration pattern was observed. In all the GBH groups bark regeneration were faster by harvesting method III followed by method II and I completing in 18 months. Bark regeneration percentage with respect to GBH, bark harvesting methods and time taken for regeneration is represented in Table 1.

Table 1: Bark regeneration percentage with respect to GBH, blaze size, and time in *Holarrhena antidysenterica*

GBH group (cm)	Method	Bark regeneration %		
		6 months	12 months	18 months
10-40	I	40.33±1.25b	44.27±1.14c	14.78±0.82a
	II	41.35±0.36b	46.64±0.20b	11.51±0.09b
	III	45.62±0.49a	48.86±0.51a	5.63±0.06c
41-80	I	35.33±0.47c	41.66±1.24c	25.66±1.69a
	II	39.25±1.69b	45.15±0.34b	15.09±0.23b
	III	41.93±0.26a	47.62±0.41a	9.76±0.35c
81-120	I	32.47±0.17c	37.85±0.37c	29.53±0.64a
	II	35.24±0.45b	40.57±0.74b	23.87±0.43b
	III	39.85±0.73a	46.27±0.64a	13.57±0.27c

Mean values within each column for a Girth at breast height (GBH) group followed by different letters differ significantly at $P \leq 0.05$



Graph 1: Bark regeneration percentage respect to GBH, blaze size, and time in Holarrhena antidysenterica

Significant difference in bark regeneration was observed with respect to different harvesting season. The data revealed that the bark regeneration was faster when the harvest was done in the month of March followed by December, irrespective of girth classes. In smaller girth classes, i.e., 10−40 cm and 41−80 cm bark regeneration was faster than 81−120 cm girth and completing bark regeneration in 18 months. The affect of harvesting season on bark regeneration in Kutuj is represented in Table 2.

Table 2: Seasonal variation in bark regeneration of *Holarrhena antidysenterica*

GBH group (cm)	Harvesting seasons	Bark regeneration %		
		6 months	12 months	18 months
10-40	March	43.59±0.29a	48.23±0.54a	7.98±0.09d
	June	30.25±0.54c	45.63±0.52b	23.96±0.10a
	Sept.	40.56±0.63b	44.65±0.23c	15.63±0.53b
	Dec.	41.63±0.23b	45.96±0.12b	11.98±0.36c
41-80	March	41.86±0.52a	47.96±0.37a	11.98±0.36c
	June	30.21±0.23d	46.63±0.68b	23.12±0.12a
	Sept.	34.79±0.48c	46.53±0.57b	18.63±0.16b
	Dec.	38.64±0.52b	45.67±0.42c	15.63±0.21c
81-120	March	38.98±0.89a	45.79±0.77a	14.96±0.15d
	June	29.23±0.46d	42.63±0.23c	27.85±0.62a
	Sept.	31.97±0.50c	41.52±0.41d	25.76±0.34b
	Dec.	35.48±0.61b	43.52±0.35b	20.65±0.53c

Mean values within each column for a GBH group followed by different letters differ significantly at $P \leq 0.05$

The data revealed significant variation in total phenols and tannins with regard to different GBH groups. Total phenols were found maximum (9.21±0.32%) in 81−120 cm GBH group in March followed by 8.61±0.25% in 41-80 cm GBH group in March and minimum (3.12±0.05%) in 10-40 cm GBH group in June. Total flavonoids were found maximum (0.30±0.08%) in 81−120 cm GBH group in March followed by 0.29±0.11% in 41−80 cm GBH group in March and minimum (0.05±0.01%) in 10−40 cm GBH group in June. Tannins were found maximum (9.89±0.05%) in 81-120 cm GBH group in March followed by 9.74±0.17% in 41−80 cm GBH group in March and minimum (4.89±0.04%) in 10−40 cm GBH group in June. Total alkaloids content was observed maximum (3.11±0.10%) in 81−120 cm GBH group in March

followed by 2.96±0.09% in 41−80 cm GBH group in March and minimum (1.11±0.07%) in 81−120 cm GBH group in June. Total phenols, total flavonoids, tannins, and total alkaloids content of *H. antidysenterica* bark are presented in Table 3.

Table 3: Total phenols, total flavonoids, tannins and total alkaloids content in *Holarrhena antidysenterica* bark

GBH group (cm)	Harvesting season	Total phenols %	Total flavonoids %	Tannins %	Total alkaloids%
10-40	March	6.48±0.12a	0.16±0.08a	7.65±0.16a	2.36±0.05a
	June	3.12±0.05c	0.05±0.01c	4.89±0.04c	1.13±0.01c
	Sept.	3.54±0.09d	0.09±0.03d	5.45±0.07d	1.49±0.01d
	Dec.	4.86±0.15b	0.14±0.05b	6.98±0.12b	2.11±0.03b
41-80	March	8.61±0.25a	0.29±0.11a	9.74±0.17a	2.96±0.09a
	June	4.25±0.12c	0.11±0.02c	5.28±0.10d	1.21±0.05c
	Sept.	4.96±0.17c	0.19±0.08c	6.98±0.13c	1.85±0.09c
	Dec.	7.63±0.21b	0.24±0.14b	7.54±0.20b	2.46±0.12b
81-120	March	9.21±0.32a	0.30±0.08a	9.89±0.05a	3.11±0.10a
	June	4.65±0.08d	0.05±0.02d	6.12±0.08c	1.11±0.07c
	Sept.	6.52±0.14c	0.15±0.07c	6.21±0.11c	2.05±0.15b
	Dec.	8.04±0.13b	0.21±0.05b	7.98±0.09b	2.86±0.06b

Mean values within each column for a GBH group followed by different letters differ significantly at $P \leq 0.05$

On analyzing different plant parts for total phenols, total flavonoids, tannins and total alkaloids content it was found that they were maximum (7.51±0.12%, 0.19±0.09%, 8.61±0.10%, and 2.25±0.06%) in trunk bark followed by leaves (3.18±0.05%, 0.07±0.02%, 3.80±0.07%, and 1.21±0.03%) and minimum in flowers (0.12±0.02%, 0.02±0.01%, 0.26±0.02%, and 0.11±0.02%). It clearly indicates that all phytochemical contents were having more concentration in trunk bark in comparison to other plant parts as represented in Table 4.

Table 4: Total phenols, total flavonoids, tannins and total alkaloids content in different plant parts of *Holarrhena antidysenterica*

Samples	Total phenols %	Total flavonoids %	Tannins %	Total alkaloids %
Trunk bark	7.51±0.12a	0.19±0.09a	8.61±0.10a	2.25±0.06a
Leaves	3.18±0.05b	0.07±0.02b	3.80±0.07b	1.21±0.03a
Twig bark	0.34±0.07c	0.05±0.03bc	0.59±0.05c	0.78±0.06b
Wood	0.15±0.03cd	0.03±0.01c	0.83±0.06c	0.23±0.04b
Flowers	0.12±0.02d	0.02±0.01c	0.26±0.02c	0.11±0.02b

Mean values followed by different letters differ significantly at $P \leq 0.05$

The results revealed that bark regeneration was faster in younger and middle aged trees which corroborates with the findings that trees of *Pseudocedrela kotschyi* had similar pattern, i.e., medium sized trees (21–30 cm dbh) had a faster bark recovery than the other studied dbh classes (Delvaux *et al.* 2010) [16].

Strip harvesting (method III) showed faster bark regeneration in all the GBH groups in both the species because in this method only small portion of bark was removed, resulting in smaller wound. Moreover, bark regeneration was also faster during initial 6 months due to plant wound closure mechanisms that occur during initial few months after wounding (Schmitt and Liese, 1993, Oven and Torelli, 1994) [17, 18]. In trees of higher girth classes and large blazes the size of wound is bigger resulting in more exposed surface area, which takes long time to recover. In first 6 months after harvest mostly edge growth was observed during bark regeneration process there after both edge and sheet growth were observed that are in accordance to the findings that were reported edge and sheet bark regrowth in some medicinal trees species of Benin, West Africa (Delvaux *et al.* 2009) [19]. This could be explained by a higher hormonal activity stimulated by stress in order to restore water conductivity and thus to close the wound as soon as possible (Mohr and Schopfer, 1995) [20].

Bark regrowth varies with site differences, season and microclimate. It is reported that bark regrowth varied with site differences, season, and microclimate (Cunningham, 2001) [21]. It also reported that response to bark stripping could be affected by season of harvesting and this varies between species (Geldenhuys *et al.* 2002 and Vermeulen and Geldenhuys, 2004) [22, 23]. The present study revealed that on the basis of quality of the bark with respect to their phytoconstituents, the bark harvested in the month of March has higher concentration of constituents as compared to the bark harvested in the month of June. Therefore ideal time for bark harvesting to get quality produce is from February to March for the species.

Conclusion

H. antidysenterica (Kutaj) bark can be obtained on sustainable basis if the bark is harvested through non-destructive harvesting techniques and sufficient time is allowed between two successive harvests for the plant to regenerate new bark. Bark should be harvested longitudinally, not all over the circumference of trunk and branches. In younger trees having GBH less than 30 cm bark should be extracted by removing 5−6 cm wide strips from the main trunk of the tree. For sustainable harvest, strip harvesting should be done on the tree trunk. Only outer and middle bark should be removed leaving the inner bark for regeneration.

Sustainable bark harvesting can be done after every 18 months by extracting opposite strip of the trunk bark. Sustainable bark harvesting techniques should be practiced in order to conserve and sustainably utilize the resources.

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