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Detection of Phytochemicals in the Different Extracts of *Dhaura*, *Anogeissus Acuminata* (Roxb.) Wall. Ex Bedd

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Abstract

Several plants are being used to treat different ailments in humans since time immemorial and *Dhaura* or *Anogeissus acuminata*, or button tree is no exception. Its use has been indicated in several medicinal folklore systems in India to cure several diseases. The present study was undertaken to evaluate the different Phytochemicals present in the whole stem bark as well as different extracts of the stem bark of *dhaura*. The Phytochemical analysis of whole stem bark has shown that it contains phenols, flavonoids apart from carbohydrates, proteins and amino acids. However, alkaloids, cardiac glycosides and tannins were absent. The analysis of chloroform, ethanol and aqueous extracts showed that the chloroform and ethanol extracts contained alkaloids, flavonoids, cardiac glycosides, phenols and tannins apart from proteins and amino acids, whereas, carbohydrates were completely absent. The aqueous extract did not show presence of alkaloids and tannins.

Keywords: *Anogeissus Acuminata;* Phytochemical; Flavonoids; Cardiac glycosides; Alkaloids

Introduction

The nature has provided several varieties of plants and many of the flowering plants have been or are being used as medicine since time immemorial. The plants and other natural products have formed the basis of traditional, folklore and other systems of medicine. In fact, application of plants as medicinal entities is documented in Vedic times in the Rig-Veda, the oldest text, which mentions the use of 107 plants for medical purposes [1]. The Atharva Veda is devoted to diseases and their cure using plants and herbs and the ancient system of medicine the Ayurveda is an shoot of this epic of Hindus [2]. In the modern era chemical molecules forms the basis of modern allopathic system, which has also relied on plants to isolate many drugs before their actual chemical synthesis was undertaken.

The evolution of modern medicine has put the use of plant and natural products-based therapy in dormancy for some time, especially for two hundred years. Nevertheless, use of traditional medicine is still preferred by 80% of the global population for healthcare [3]. However, there has been a resurgence of use of plant and

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natural products-based therapy in the late 20th century, which is indicated by a 380% increase in their use, especially in the United States of America [4]. This shift may be due the realization that use of antibiotics and synthetic drugs develop resistance to therapy and it is difficult to get the chronic diseases cured with modern therapy [5,6]. This has given impetus to rediscover and scientifically establish the use of plants and natural products as medicine.

Anogeissus acuminata or *Dhaura* (Hindi) is a deciduous tree consisting of a narrow crown; and grows up to 40 meters tall. Its long, straight bole is unbuttressed and can reach 100 cm in diameter. It is found in tropical and semi deciduous regions of India, Bangladesh, Myanmar, Thailand, Cambodia, Laos, Vietnam, Arabian Peninsula and Africa [7]. The medicinal value of *dhaura* has been described in Ayurveda, where its roots are considered as bitter, acrid, cooling, vulnerary and alexipharmic. The use of roots is indicated in the treatment of leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensations, asthma, leukoderma, fatigue and blood diseases. Decoction of root is used with water to gargle to reduce toothache. *Dhaura* is very useful in diarrhoea, amoebic dysentery, bleeding piles and urinary infections. It is a good medicine to cure skin diseases and also used in conditions like bronchitis, general weakness, neurological problems and impotence. It is useful in the treatment of spleen enlargement, dysuria, cold, cough, cholera, excessive perspiration, urinary disorders, in scorpion sting and snake bite. Bark is bitter, astringent. The gum (called Ghatti or Indian gum) is used as a tonic after delivery. Gum gives a stable oil-in-water emulsion and patented for incorporating oil-sol, vitamin preparations and has excellent emulsifying properties [8]. It has been reported to be active against the alloxan-induced diabetes in rats [9]. The hydroalcoholic extract of stem bark and leaves of *dhaura* has been found to be antioxidant in vitro and Hepatoprotective in Wistar rats [9]. Therefore, the present study was undertaken to detect various Phytochemicals in the stem bark of *dhaura* i.e. *Anogeissus acuminata.*

Materials and methods

Extraction of stem bark

Dhaura or *Anogeissus acuminata* (family: Combretaceae) or button tree *Anogeissus pendula* Edgew. *Conocarpus acuminatus* Roxb. ex DC. was identified by the Department of Horticulture Aromatic and Medicinal Plants, Mizoram University, Aizawl, India. The non-infected stem bark of mature tree of *dhaura* was carefully peeled off from the Mizoram University campus in the months of September to December. The bark was thoroughly washed with clean water so as to disengage dust and other unnecessary extraneous material and shade dried. The stem bark of *dhaura* was chopped into small pieces and powdered in an electrical grinder. The dried powder of *dhaura* was sequentially extracted in petroleum ether, chloroform, ethanol and water using Soxhlet apparatus.

Phytochemical analysis

The analysis of different Phytochemical constituents in the stem bark, and its chloroform, ethanol and water extracts were performed as described below:

Alkaloid assay: The test for the presence of alkaloids was done by dissolving 0.5 g of sample in 10 ml of acidified alcohol, followed by boiling and filtering. The 5 ml of the filtrate was mixed with 2 ml of dilute ammonia and 5 ml of chloroform. The mixture was shaken gently to extract the alkaloidal base. The chloroform layer was further extracted with 10 ml of acetic acid and divided into two parts. Mayer's reagent was added to one part, whereas Draggendorff's reagent to the other. The formation of a cream (Mayer's reagent) or reddish-brown precipitate (Draggendorff's reagent) indicated the presence of alkaloids [10,12].

Flavonoid assay: The presence of flavonoids in *dhaura* was determined by different methods. The aqueous sample filtrate was mixed with 5 ml of dilute ammonia solution with the addition of concentrated H_2SO_4. Development of yellow color indicated the presence of flavonoids. A few drops of 1% aluminum solution were added to a portion of sample filtrate. A yellow color indicated the presence of flavonoids. The sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow color indicated the presence of flavonoids [10,12].

Lead acetate test: The different extracts of *dhaura* were treated with a few drops of lead acetate solution. Formation of yellow precipitate indicated the presence of flavonoids [10,12].

Cardiac glycosides

Keller Killani test was performed to test the presence of cardiac glycosides, where 5 ml of sample was mixed with 2ml of glacial acetic acid containing one drop of ferric chloride. This was underlaid with 1ml of conc. sulphuric acid. A brown ring of the interface indicated deoxy sugars, which showed presence of cardenolides. A violet ring may form below brown ring while in acetic acid layer, a greenish ring may form gradually throughout thin layer [10,12].

Assay for phenols: Usually 0.2 g of the sample was treated with 3-4 drops of ferric chloride solution. The formation of bluish black color indicated the presence of phenols (Ferric chloride test) [10,12].

Assay for tannins: About 5g of sample was boiled in 20 ml of double distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate. The development of brownish green or a blue-black color indicated the presence of tannins [10,12].

Assay for carbohydrates: The sample was dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Filtrates were treated with Benedict's reagent gently. Orange red precipitate indicated the presence of reducing sugars [10,12].

Biuret assay for proteins and amino acids: To the alkaline filtrate of the sample (0.5 % w/v solution of test residue), 2-3 drops of copper sulphate solution were added. Presence of red violet color indicated the presence of proteins and free amino acids [10,12].

Results

The results of different Phytochemicals present in the *dhaura* are indicated in Tables 1-4.

The analysis of different Phytochemicals in the whole stem bark of *dhaura* did reveal the presence of phenols, cardiac glycosides, flavonoids (lead acetate test), carbohydrates, proteins and amino acids, whereas, alkaloids, and tannins could not be detected (Table 1).

Phytochemicals	Absence (-ve)/Presence (+ve)
Alkaloids	-ve
Cardiac glycosides	+ve
Phenols	+ve
Tannins	-ve
Flavonoids Alkaline reagent test Lead acetate	 -ve +ve
Carbohydrates	+ve
Proteins and amino acids	+ve

Table 1: Phytochemical analysis of whole stem bark of *Anogeissus acuminata.*

Assay of different Phytochemicals in the chloroform stem bark extract of *dhaura* has shown that it contained alkaloids, cardiac glycosides, phenols, flavonoids, tannins and proteins and amino acids, whereas carbohydrates were absent (Table 2).

Phytochemicals	Absence (-ve)/Presence (+ve)
Alkaloids	+ve
Cardiac glycosides	+ve
Phenols	+ve
Tannins	+ve
Flavonoids Alkaline reagent test Lead acetate	 +ve -ve
Carbohydrates	-ve
Proteins and amino acids	+ve

Table 2: Phytochemical analysis of Chloroform extract of *Anogeissus acuminata.*

The ethanol extract of stem bark of *dhaura* showed the presence of all Phytochemicals including, alkaloids, flavonoids, cardiac glycosides, tannins, phenols proteins and amino acids but carbohydrates were absent (Table 3).

Phytochemicals	Absence (-ve)/Presence (+ve)
1. Alkaloids	+ve
3. Cardiac glycosides	+ve
4. Phenols	+ve
5. Tannins	+ve
6. Flavonoids Alkaline reagent test Lead acetate	 +ve -ve
Carbohydrates	-ve
Proteins and amino acids	+ve

Table 3: Phytochemistry of Ethanol extract of *Anogeissus acuminate*.

The aqueous extract of stem bark of *dhaura* consisted of various Phytochemicals like, cardiac glycosides, flavonoids (lead acetate test), phenols, carbohydrates, proteins and amino acids. However, alkaloids and tannins were completely absent (Table 4).

Phytochemicals	Absence (-ve)/Presence (+ve)
Alkaloids	-ve
Cardiac glycosides	+ve
Phenols	+ve
Tannins	-ve
Flavonoids Alkaline reagent test Lead acetate	 -ve +ve
Carbohydrates	+ve
Proteins and amino acids	+ ve

Table 4: Phytochemical analysis of aqueous extract.

Discussion

The plants synthesize different primary and secondary metabolites to meet their day to day metabolic needs and to defend and protect themselves from different types of environmental stresses. The primary metabolites are utilized for growth and other vital functions for the sustenance of plants. The secondary metabolites may not be really necessary for survival of the plants but they play other important roles secondary to plant survival. The secondary metabolites are of great use for humans. Therefore, the present study was carried out to analyze the different Phytochemicals present in the *Anogessius acumiata* or *dhaura*.

The *dhaura* has been found to contain alkaloids, especially in chloroform and ethanol extracts. Similarly, alkaloids have been detected in the methanol extract of bark and leaves of *dhaura* earlier [13]. The alkaloids are secondary metabolites synthesized by several plants and they basically contain nitrogen atom in a heterocyclic ring structure. The alkaloids are particularly synthesized by higher plants, which are known to synthesize more than 12000 alkaloids. The alkaloid containing plants have been used as medicine, poisons and narcotics since ancient times [14]. These plant secondary metabolites possess diverse pharmacological properties and they find immense use in medicine for human healthcare. They are used as anesthetics, antioxidants, anti-inflammatory agents, stimulant, and in the treatment of several diseases including cancer [15-18]. The plant alkaloids are products of their metabolic activities and they play important role in nitrogen storage, protection against predation, and also regulate plant growth [19,20].

The other important classes of Phytochemicals synthesized by plants are flavonoids. The plants synthesize more than 10,000 flavonoids. They are nonessential secondary metabolites; however, they play crucial role in growth regulation, interaction of plants with their environment, protection from harmful UV radiation and sexual reproduction [20,22]. The colors of flowers and fruits is due to flavonoids and many of them serve as co pigments causing different colors of flowers [23,24]. Flavonoids also act as defense weapon against invading phytopathogens [25]. The antioxidant activity of flavonoids is well documented in plants as well as humans [26]. The flavonoids have been reported to have several activities in human beings including antiviral, anti allergic, antibacterial, antisteoporotic, anticancer, cardio-protective Hepatoprotective, anti-inflammatory, anti-catatactogenic, and antidiabetic [27-33]. Like other plants, *dhaura* also synthesizes flavonoids which have been detected in whole plant as well as chloroform, ethanol and aqueous extracts. The presence of flavonoids has been reported in *dhaura* earlier [9,34,35].

Different extracts of *dhaura* were positive for the presence of cardiac glycosides. The synthesis of glycosides has been detected earlier in the methanol extract of stem bark and leaves of this plant [13]. Cardiac glycosides are important drug candidates and their cytotoxic effects have been realized as early as 1967 in cultured cell lines [36,37]. The other cardiac glycosides frequently used in clinics is digoxin, which finds its use in combination with chemotherapy to increase the overall survival of cancer patients receiving chemotherapy for colorectal, breast, head and neck and Hepatocellular carcinoma [38]. The cardiac glycosides have been also used in the treatment of cardiovascular diseases [39].

Tannins have been detected in the methanol bark and leaf extracts of *dhaura* earlier [13]. We have also found tannins in the chloroform and ethanol extracts of *dhaura*. Tannins are highly complex polyphenolic compounds synthesized by several plants [40,41]. The tannins protect plants against the attack by herbivores and insects by decreasing the availability of proteins or inducing toxicity [41,43]. They act against microorganism invasion of plants and protect the plants due to their ability to form complexes with protein, starches and other macromolecules [44]. The tannins are antibacterial, astringent, antioxidants, antiviral, antiulcerogenic, anti tumor, antithrombogenic, and anti-inflammatory in humans [41,45-47]. However, tannins become prooxidants in the presence of oxygen [41].

The primary metabolites like carbohydrates, proteins and amino acids are usually synthesized by almost all plants as they are essential in performing several vital functions. *Dhaura* also synthesizes these primary metabolites to meet normal activities for its survival. The stem bark and leaf extracted in methanol of *dhaura* have been found to synthesize carbohydrates, proteins and amino acids earlier [13,34]. However, the carbohydrates couldn't be detected in chloroform and ethanol extracts in the present study.

Conclusions

The Phytochemical analysis of the stem bark of *Anogeisseus acuminata* i.e. *dhaura* has shown the presence of alkaloids, cardiac glycosides, flavonoids, tannins, and phenols as secondary metabolites, whereas carbohydrates, proteins and amino acids were detected as primary metabolites required for meeting daily requirements for its sustenance. The medicinal properties of *dhaura* may be due to the presence of these secondary

metabolites whose medicinal properties have been established scientifically. The presence of secondary metabolites reaffirms the ethnomedicinal use of *dhaura* in humans.

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