Pharmacognostic, Phytochemical and Biological investigations of *Bauhinia variegata* (L). fruit.

Sidra Muhammad Ali[1], Adeel Usman[2*], M.Asif Wazir[2],Talal Shaheer[2], Rizwana Manzoor[1,] and Khizar Abbas[1*]

[1]Department of Pharmacognosy, Faculty of Pharmacy,
Bahauddin Zakariya University, Multan, Pakistan.
[2]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences,
University of Karachi, Karachi, Pakistan.

Corresponding author: Adeel Usman
Email address: Adeelusman99@yahoo.com , **Cell:** +92-0333-7640989

Abstract

Plants have been constantly utilized as medication by human beings to maintain healthy state and treat mankind suffering from illnesses and still main stream to acquire new variety of cosiness and medication as well. The present study was designed to validate the pharmacognostic, phytochemical and biological evaluation of *Bauhinia variegata* L. fruit. *Bauhinia Variegata* L. is a genus of flowering herbs in a legume family Fabaceae which is a rich source of nutritional and medicinal agent. Powder microscopy was carried out with chloral hydrate, phloroglucinol and glycerin for identification after observing organoleptics of plant (i.e color, odor, taste) of cellular fragment, anatomical and diagnostic feature through powder microscopic examination. Fluorescence analysis revealed different colors both in visible and UV light with different reagents. Phytochemical screening was performed for the presence of different class of compounds with different chemical/reagents. Antibacterial assay was conducted by using disc diffusion method, ciprofloxacin was used as standard drug against *E. coli, S. aureus, B. subtitis and S. maltophilia*. The results depicted that powder microscopy reveals trichomes, calcium oxalate crystals, xylem vessels, and epidermal cells with anomocytic stomata while phytochemical evaluation of the crude extracts of *Bauhinia variegata* fruit showed the presence of alkaloids, flavonoids, phenolic compounds, tannins, quinones, terpenoids, and glycosides. Fluorescence analysis revealed different colors both in visible and UV light with different reagents. MeOH extract (both boiled and un-boiled) of the *Bauhinia variegata* L. fruit demonstrated a significant antibacterial effect. The plant material revealed prominent antioxidant activity, ciprofloxacin was used as standard drug. The plant extract also showed significant spasmolytic potential on rabbit jejunum. This research represents a detailed survey of literature on plants profile, phytochemistry and pharmacological activities of *Bauhinia variegata* L.

Keywords:

Bauhinia variegata, Antibacterial, Pharmacognostic, Spasmolytic and Antioxidant.

1. INTRODUCTION

Curing with medicinal plants is as old as humankind. The relation between man and his search for herbs in nature is old. Perception of medicinal herbs usage is a result of numerous years struggle against illness due to which humans become proficient to pursue herbs in seeds, fruit bodies, barks, and other parts of the herbs (Petrovska, 2012). Many botanical medications contain healing bioactive chemical elements which have proven to be valuable as primary or supplemental treatments when carefully applied (Halberstein, 2005). The importance of medicinal plants in conventional healthcare practices, providing intimation to a new area of research and biodiversity conservation is now well acknowledged (Uniyal et al, 2006). Modern medicine rely on native system only after chemical and clinical test. Substitute drugs causes side effects as an outcome, people are more focused to use natural products acquired from plants. It has been approximated that 56% of the active compounds for medicines in the British National Formulary are proper products (Anjoo, K et al. 2011).

The exploration for new molecules, nowa days has taken a slightly different route where the science of ethnobotany and ethnopharmacognosy are being used as guide to lead the Pharmacognosist to different sources and classes of compounds (Gurib-Fakim, 2006). Current research in drug learning from medicinal herbs involves a multifaceted approach combining biological, phytochemical and molecular techniques. Medicinal plants drug discovery maintains to provide various pharmacological targets including cancer, HIV/AIDS, Alzheimer's disease, pain and malaria (Balunas, 2005). Herbal medicines have essential role in the precaution and treatment of cancer. With exceptional knowledge of molecular science and refinement in isolation and structure elucidation techniques, various anticancer herbs have been recognized. Many anticancer agents comprise of Taxol, vincristine, vinblastine, topotecan and irinotecan, camptothecin derivatives, and etoposide derived from epipodophyllotoxin are in clinical use all over the world (Manju et al, 2012).

Bauhinia variegata L. a genus of flowering herbs in a legume family of Fabaceae. It is from China Southeast Asia to the Indian subcontinent. Usually names called orchid tree (however not associated to the family Orchidaceae) and mountain ebony. In Pakistan it is commonly known as *Kachnar, Kachnal, Gurial.* (GRIN, Agricultural Research Service. USDA 2019). Indigenous traditional system of medicines. It is an edible plant, many portions of the plant are used as anthelmintic and anti-microbial, anti-leprotic, astringent, liver tonic and in the treatment of dysmenorrhea. *Bauhinia variegata* L. is also useful for treatment of skin diseases, dysentery, ulcers, wounds, edema, eye disease, piles, hemorrhoids and an Antidote against snake bite (Qaisar, M, et al. 2012). *Bauhinia variegata* L. contains alkaloids, lupeol, phenolic, lignin, saponins, oil, fat, glycoside, terpenoids, â-sitosterol, rutin, quercetin, apigenin, kaempferol-3-glucoside, tannins, apigenin-7-o-glucoside, amides, carbohydrates, decreasing sugar, phosphorus, protein, fiber and vitamin C with calcium.(Fig-1). The traditional uses of the plant do not reveal a scientific proof for the clinical use of the plant. That is why the present study was designed to scientifically evaluate the biological potential of the plant..

Fig-1 *Bauhinia variegata* L.
(Source: coutesy of.1mg.com/ayurveda/kachnar-212.)

2. MATERIAL AND METHODS

2.1. Collection and authentication of the plant.

Bauhinia variegata L. was purchased from the market in Multan in the month of April 2019, and it was authenticated as *Bauhinia variegata* L. (Family, Fabaceae) by a taxonomist Dr. Zafar Ullah Zafar (Associate Professor, Department of Botany, Bahauddin Zakariya University Multan) under Voucher No. R. R. Stewart F.W. Pak.347 (9). Fruit was separated, shade dried for 40 days at room temperature and ground to a coarse powder.

2.2. Extract preparation.

Fruit of B.*Variegata* L. (kachnar) was taken almost 2.0 kg. 1.0 kg fruit was boiled with enough amount of water for 30 minutes. After filtration, aqueous filtrate was freeze dried by using lyophilizer and the marc was macerated in methanol for a period of 7 days with occasional shaking. fiterate was concentrated by using rotary evaporator. Other 1.0 kg of plant was macerated in methanol, occasionally shaken during maceration followed by filtration.The semisolid extracts obtained by successive extraction was weighed and stored in amber glass containers. Now, we have three samples named as boiled (methanolic), un-boiled (methanolic) and lyophilized sample.

2.3. Fluorescence analysis

Florescence analysis of flowers of the plant was performed according to the standard protocol described in literature (Chase and Pratt, 1949).

2.4. Powder microscopy:

These studies of the fruit of plant were performed according to the standards protocol enlisted by World Health Organization (WHO 2011).

2.5. Phytochemical evaluation:

Phytochemical screening for the presence and absence class of chemical compounds like alkaloid, tannins, quinones, saponins, phenol, flavonoids, terpenoids, and glycosides was performed according to the standard procedure described earlier by Brain and Turner (Brain and Turner, 1975).

2.6.Antibacterial activity:

The antibacterial activity of the fruit of *Bauhinia variegata* L. was assessed using a process which is called disc diffusion method. Take NA almost 28 g dissolved water in it 1000 ml and shake well. This media is sterilized for 15 minutes by the use of autoclave under temperature 121 °C with pressure of 15 psi. Firstly, prepared media was poured into the petri dish then allowed it to solidify. Then streak the agar media with bacterial strain using spreader in such a way that agar plate was completely inoculated with bacterial strain and the three soaked 6mm disc of extract desired concentration, disc infused with Ciprofloxacin (Positive control) and disc infused with DMSO (negative control) were placed in inoculated plate at equal distance from each other. Then incubate at 37 °C for one day. Then determined minimum inhibitory concentration which inhibits bacterial growth.Then the dilution of varying concentration at 200 mg, 400 mg and

600 mg were tested by the same method and zone of inhibition around the sample and control discs were measured in mm by using a ruler. This assay was performed thrice (Egbuonu & Osuji, 2016).

2.7. Antioxidant assay:

Total assay volume was 100µl, containing 10ìl, of the test solution (10 mg/ml) and 90 ìL of DPPH (100 µM) in methanol solution in a 96 well plate.

The contents were mixed and incubated at 37°C for 30 minutes. Synergy HT BioTek® USA microplate reader was used to determine the decrease in absorbance at 517nm. Standard antioxidant was ascorbic acid (0.5 mmol). All experiments were carried out in triplicate. The decrease in absorbance indicated increased radical scavenging activity which was determined by the following formula.

Percentage inhibition= (a-b)/a × 100

Where, a = Absorbance of control or standard, b= Absorbance of sample solution (Ratshilivha *et al*, 2014).

3. RESULTS AND DISCUSSION

The *Bauhinia variegata* L.is the outmoded plant which is used for great medicinal potential such as hepatoprotective, antibacterial, anti-inflammatory, nephro-protective, hypolipidemic, antioxidant, wound healing, antiulcer, molluscicidal, dysentery, antidiabetic, diarrhea, piles, edema, anthelmintic, laxative, astringent, anti goitrogenic, antileprotic, antitumor, in dyspepsia, antidote for snake poisoning, and carminative etc.

It is particular of the traditional value in therapeutic number of diseases has been established by a pharmacological screening of numerous parts of this plant. Microscopic evaluation method is used to identify the drug on the cellular basis.It is used to determine the structure of organized drug by histological characters such as shape,size,position of cells, tissues and the likes.. In the present study crude drug powder was used for microscopic evaluation showed the presence of 142.8- 199.92 ìm long multicellular uniseriate trichomes, 57.12 ìm in diameter prismatic calcium oxalate crystals, lignified annular and spiral xylem vessels, portion of epidermal cells with anomocytic stomata (**Fig-2**).

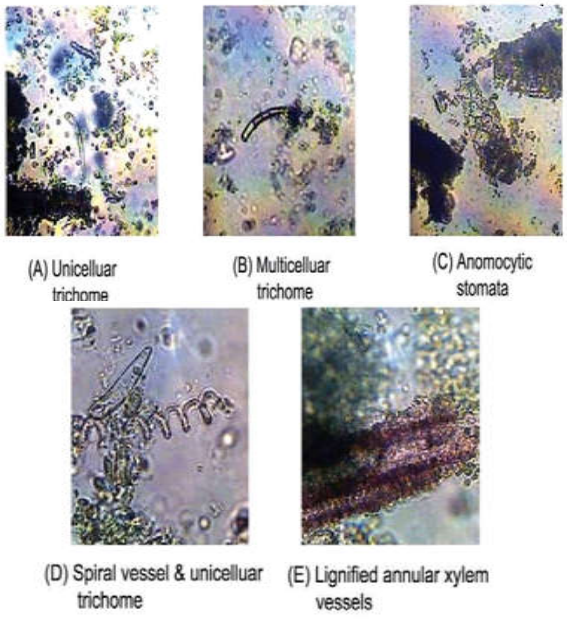

Fig-2 Powder microscopy of *variegata* L.

Fluorescence analysis is carried out to establish the ingenuity of plant drugs. The fluorescence color is specific for each part when treated with different solvents and observed under day light and UV lamp. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g.

alkaloids like berberine), which do not visibly fluorescence in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by treating with different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (**Table.1**). (Gupta et al, 2006).

Table 1. Fluorescent analysis of fruit of *Bauhinia variegata* L.

Sr. No	**Organic solvent**	**Visible light**	**Fluorescence in UV light**	
			254nm	**366nm**
1	Distilled water	Cream	Cream	Cream
2	Acetone	White	White	Brown
3	Ethanol	White	White	Green
4	Benzene	Mud brown	Light brown	Dark Brown
5	Chloroform	Mud brown	Light brown	Brown
6	Diethyl ether	White	White	Cream
7	Methanol	White	White	Cream
8	Petroleum ether	White	White	White
9	Sulphuric acid	Reddish	Reddish brown	Brown
10	Nitric acid	Brown	Reddish	Brown
11	$FeCl_3$	Brown	Mustard yellow	Brownish Black

Phytochemicals are natural substances present only in plants; they do not constitute the direct part of the plant but are of great medicinal importance because they contain bioactive compounds of targeted potential.In the present work phytochemical screening of the crude drug showed the presence of phytochemicals like alkaloids,tannins,quinines,phenols,saponins, flavonoids, terpenoids and glycosides present in (**Table.2**).

Table 2. Phytochemical screening of methanolic extract of *Bauhinia variegata* L. fruit.

Sr.No.	Phyto-constituents	Test performed	Observation	Result(+,-)
1	Alkaloids	Mayer's	Cream precipitates	+
		Wagner's	Reddish precipitates	+
		Ferric Chloride	Yellow precipitates	+
		Hager's	Bright yellowprecipitates	+
		Dragendroff's.	Orange precipitates	+
		Tannic Acid.	No precipitates	-
2	Tannins	FeCl3.	Blackish precipitate	+
		Gelatin.	White precipitate	+
3	Quinones	NaOH.	Color ranging from blue to red.	+
		HCl.	Black color.	+
4	Saponins	Foam.	No foam formation.	-
5	Phenol	FeCl3.	Blackish precipitate	+
		Alkaline reagent.	Red to yellow color formation	+
6	Flavonoids	Lead acetate.	Red precipitate formation	+
		Sulphuric acid.	No precipitates	-
7	Terpenoids	H2SO4	Red precipitates	+
		Bromine	Pale yellow color	+
8	Glycosides	Molisch's	Reddish purple ring	+
		Keller Kiliani	Reddish brown ring at the junction.	+

Table 3a. Antibacterial activity of boiled methanolic extract of *Bauhinia variegata* L.

Media mg/ml	**Inhibition zone.**					
	Escherichia. Coli.	*Staphylococcus Aureus.*	*Bacillus subtilis.*	*Bacillus megaterium*	*Steno. maltophilia*	*Serratia marescens*
MIC. mg/ml	70	100	100	100	-	100
200. mg/ml	6 ±0.2	12 ±0.3	6 ±0.7	-	-	7.6 ±0.6
400. mg/ml	11 ±0.65	16 ±0.4	8 ±0.6	-	-	11 ±0.15
600. mg/ml	13.7±0.1	17 ±0.5	11 ±0.6	-	-	14 ±0.63
Ciprofloxacin	15	19	16	14	17	22

Table 3b. Antibacterial activity of un-boiled methanolic extract of *Bauhinia variegata* L.

Media mg/ml	**Inhibition zone.**					
	Escherichia Coli.	*Staphylococcus Aureus.*	*Bacillus subtilis.*	*Bacillus megaterium.*	*Steno. maltophilia*	*Serratia marescens*
MIC. mg/ml	100	100	75	-	-	-
200. mg/ml	7 ± 0.88	6 ± 0.42	6.4 ± 0.43	-	-	-
400. mg/ml	11 ± 0.72	8 ± 0.16	10 ± 0.36	-	-	-
600. mg/ml	13 ± 0.11	11±0.41	12 ± 1.88	-	-	-
Ciprofloxacin	20	17	23	18	19	22

Table 3c. Antibacterial activity of freeze dried methanolic extract of *Bauhinia variegata* L.

Media mg/ml	**Inhibition zone.**					
	Escherichia Coli.	*Staphylococcus Aureus.*	*Bacillus subtilis.*	*Bacillus megaterium.*	*Steno. Maltophilia*	*Serratia marescens*
MIC. Mg/ml	100	100	100	-	-	50
200. mg/ml	7.74 ± 0.02	7±0.11	5.4 ± 0.13	-	-	8 ± 0.16
400. mg/ml	8.51 ± 0.03	9.22 ±0.12	7.6 ± 0.04	-	-	11 ± 0.21
600. mg/ml	14 ± 0.12	12.51±0.17	11.65 ±0.11	-	-	13 ± 0.14
Ciprofloxacin	26	22	21	18	14	21

Antibacterial activity is the most important characteristic of medical textiles, to provide adequate protection against microorganisms, biological fluids, and aerosols, as well as disease transmission. Antibacterial activity can be defined as a term for the active principles (agents) that inhibit the growth of bacteria. In the current research work three different samples named boiled, un-boiled and freeze dried obtained by MeOH extraction were used to evaluate antibacterial potential of the plant. Results in (**Table.3a-3c**) showed a significant antibacterial potential of *Bauhinia variegata* L.The oxidation process is natural in vitality management of every single living creature.

The antioxidant activity was measured in in methanol and freeze dried extracts of *Bauhinia variegata* L. DPPH is a purple colored free radical compound, known as 2,2-diphenyl 1-picrylhydrazyl, which turns to yellow if the plant contains antioxidant. Diarrhoea is a gastrointestinal disorder that has been traditionally treated with herbal medicines all over the world. The results show that when boiled extract was administerd at 0.01mg/ml it shows a decline in graph which is more significant at 3mg/ml dose.

The methanol and freeze dried extracts showed prominent antioxidant activity as compared to the standard Ascorbic acid. (**Table 4**).

Table 4. The antioxidant activity of boiled,un-boiled (methanolic) and freeze dried extract of fruits of *Bauhinia variegata* L.

Sr. No	Extract	Conc. mg/ml	Absorbance 517nm	RSA %	Standard drug	RSA %
1	Boiled	5	0.125 ± 0.4	65%	Ascorbic acid (0.5 mmol)	80%
		10	0.121 ± 0.4	68%		
		20	0.111± 0.2	70%		
2	Un-boiled	5	0.175 ± 0.11	45%		
		10	0.135 ± 0.3	60%		
		20	0.132± 0.02	68%		
3	Freeze dried	5	0.271 ± 0.03.	35%		
		10	0.180 ± 0.01.	50%		
		20	0.138 ± 0.02.	62%		

4. CONCLUSION

It has been concluded that *Bauhinia variegata* L. possess a dinstinctive histological characters, necessary phytochemical constituents, also possess significant antibacterial and prominant antioxidant and anti-diarrhoeal potential.

Conflict of interest:

The author has no conflict of interest.

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