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"PHYTOCHEMICAL STUDIES OF SOME SPECIES OF GENUS *BAUHINIA* L."

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ABSTRACT :

This review of available literature pertaining to Ayurveda Medicobotany and Ethnobotany to understand use value of Bauhinia L. It numerous medicinal properties like anti-inflammatory, anti-diabetic, sedative, anti-parasitic, digestive, expectorant, chronic dysentery, diarrhea, ulcer, goiter, skin diseases, analgesic, antipyretic, antitumor, antioxidant and antimicrobial.

Extracts revealed the presence of Tannin, flavonoids, saponins, and phenols. Tannin content was found maximum in Bauhinia roxburghiana Viogt. leaves as compare to the other species. Bauhinia roxburghiana Viogt. Methanol extract show highest Antioxidant activity whiles the Bauhinia blakeana Dunn. has the lowest activity.

KEYWORDS : *Ayurveda Medicobotany and Ethnobotany , anti-parasitic, digestive, expectorant.*

INTRODUCTION

About 80,000 species of plants are utilized for treating various diseases in different systems of Indian medicine. As per the WHO (World Health Organization) report 80% of the world population, presently use herbal medicine for some aspects for primary health care (Sujatha, 2005). The herbal Industries, however, use not more than 500 plant resources of which 200 resources are commonly use and in major quantities. The resources, therefore, are becoming endangered because of over exploitation. The search for underutilized lesser known resources mentioned in Indian Materia Medica or found in Ethenomedicobotany literature has become essential as alternatives. Considering these fact, now a days there is growing interest in the evaluation of various plants use in Indian system of Medicine as well as ethenobotany for their medicinal properties. In some Asian and African countries, 80% of the population depends on traditional medicine for primary health care. In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (WHO, 2008).

Bauhinia L. Species named as such after Jean Bauhin and Gaspard Bauhin (Houghton, 2009). It is a medium sized deciduous tree found all over India it belongs to the family Fabaceae. It is a tree with white butterfly-like flowers, moderate evergreen. It is used in several traditional medicine systems to cure various diseases and have tremendous horticultural and ornamental potential, and are commonly used as garden shrubs or pot plants due to their exuberant flowers; cut flowers make an attractive bouquet with an estimated life- span of a few days without any special treatment. (Jaime, 2013) In Nepal, the flowers are use in curries and as fodder for cattle (Singh, *et al.,* 2012).

The medicinal value of the plant is due to the presence of various bioactive chemical constituents such as alkaloids, tannins, flavanoids and phenolic compounds (Hill, 1952). Plants belonging to the genus *Bauhinia* L. are reported to be important antidiabetic

agents; the leaves and stem-bark of these plants are used in different phytopreparations to lower blood glucose levels (da Silva and Cechinel-Filho, 2002; Cavalcanti and Favoreto, 2005; Mali *et al.*, 2007).

Bauhinia L. plant possesses antibacterial, anti-diabetic, analgesic, anti-inflammatory, anti-diarrheal, anti-cancerous and thyroid regulating activity (Indiradevi, 2015). Plants belonging to the genus *Bauhinia* L. are frequently used in folk medicine to treat infectious Diseases and several experimental studies have confirmed their antimicrobial potential, especially against pathogenic fungi and bacteria. Several pharmacological activities have been reported for many *Bauhinia* L. Species, including Hepatoprotective, antioxidant, Anti-inflammatory and anti hyperlipidemic effects (Al-Sayed *et al.,* 2014). No studies have so far been conducted on the chemical constituents and the pharmacological activities of *Bauhinia roxburghiana* Voigt., *Bauhinia hookeri* F.Muell and *Bauhinia blakeana* Dunn.

The first objective of this study was to determine the phytochemical and antioxidant studies of the leaves.

MATERIALS AND METHODS

Plant Material Collection:

Fresh and healthy plant material was collected from Agharkar Research Institute. These three species was identified with the help of flora of Maharashtra State and Flora of Bombay Presidency.

Preparation of extractives

The fine powder of plant material was obtained by passing it through mesh. 2 gm of powder was taken in a conical flask and 50 ml of solvent was added to it. The closed flask was kept on the shaker for 6 hours and allowed it to stand for eighteen hours. Then, the solution was rapidly filtered through filter paper without losing any solvent. Then the solvent was evaporated till the extract was dry. Three different types of solvent extract were prepared for further experiment i.e. Methanol, Chloroform and Distilled water.

Phytochemical analysis:

The medicinal plants are known for their various kinds of chemical constituents such as carbohydrates, cellulose, chitin, fats, proteins, starches, sugars and secondary metabolites - alkaloids, amino acids, flavonoids, glycosides, lignin, mucilage, pentoses, saponins, steroids, terpenoids and tannins etc. The detection of phytochemicals has been done qualitatively and quantitatively using various phytochemical tests.

Samples in the form of powder and different solvent extracts were used for this purpose.

Qualitative phytochemical analysis:

The qualitative microchemical analysis of leaf powders and leaf extracts was carried out using different chemical reagents as per the method described by (Trease and Evans, 1983),(Paech and Tracy, 1955), (Harbone, 1998), (Sadashivam and Manikam, 1996) and (Anonymous, 1989).

Alkaloids (Paech and Tracey, 1955)

To 9 ml of ethanol leaf extract of *Bauhinia* L. species, 1 ml of alkaloids detecting reagents (Wagner's, Hager's, and Mayer's reagents) were added in individual test tubes. The formation of the specific colour or precipitate detected the presence of alkaloids.

Following reagents were prepared using standard protocols. These reagents were used for detection of alkaloids:

- **Mayer's reagent:** 1.3 g of $HgCl_2$ and 5 g of KI were dissolved separately in 60 ml and 10 ml of distilled water respectively and both the solutions were mixed and diluted to100 ml.
  The development of cream colored precipitate on addition of extract showed the presence of alkaloids.

- **Wagner's reagent:** 1.27 g of iodine and 2 g of KI were dissolved in distilled water and diluted to 100 ml. The development of brown to reddish brown precipitate on addition of 1 ml of the reagent to 9 ml of the extract detects the presence of alkaloids.
- **Hager's reagent:** 5 ml of saturated aqueous solution of picric acid was diluted with an equal volume of water.
  The development of yellow precipitate on addition of 1ml of Hager's reagent in 9 ml of extract detection presence of alkaloids.

Carbohydrates- Molisch's test (Trease and Evans, 1983)

In 2 ml water extract 2 drops of Molisch's reagent (15% solution of ά-napthol in alcohol) and 2 drops of concentrated sulphuric acid were added. The formation of purple colored ring at the junction of two liquids indicated the presence of carbohydrate

Flavonoids (Harborne, 1973)

- **Pew's test:** Zinc Powder was added into 2-3 extract, followed by drop wise addition of conc. HCl. Formation of purple red or cherry colour indicates the presence of flavonoids.
- **NaOH test**: 2-3ml of extract and few drops of sodium hydroxide solution were added into the test tube. Formation of intense yellow colour that becomes colorless on addition of few drops of dilute HCl indicates the presence of flavonoids.
- To the portion of dissolved extract, a few drops of 10% ferric chloride solution were added. A green or blue colour indicated the presence of flavonoids.
- Lead acetate test: to the 1ml extract solution add 1ml of 10% lead acetate solution. Development of white precipitates indicates presence of flavonoids.

Glycosides (Harborne, 1998)

1 ml of ethanol leaf extract was taken in a test tube. To it one drop of water and two drops of toluene were added. In the test tube a pricate paper was suspended and test tube was firmly corked. The test tube was incubated at 40°C for 2 hrs. The change in colour of the pricate paper from yellow to reddish-brown indicated the presence of glycosides.

Proteins:

For detection of proteins different phytochemical tests were performed.

- **Biuret test (Sadashivam and Manickam, 1996)**

To 2 ml of ethanol extract, 2 ml of 10% NaOH was added and shaken. Afterwards, two drops of 0.1% $CuSO_4$ solution were added. Development of violet colour indicated presence of peptide bonds.

Starch (Paech and Tracey, 1955)

Powder of the plant material was extracted with boiling methanol. After drying, the plant tissues were centrifuged with cold water and tested with iodine solution (few crystals of iodine to 2% aq. Potassium iodide). The development of blue colour detected presence of starch.

Steroids (Trease and Evans, 1983)

For the detection of steroids following tests were performed:

Salkowaski test: 2ml chloroform and 2ml conc.H_2So_4 added to the 2ml of extract shake well. Form the layer of red chloroform, acid shows green yellow inflorescence, Indicates the presence of steroids.

Tannins (Trease and Evans, 1983)

Lead acetate test: 10% Lead Acetate added to 5ml extract. Form the yellow or red precipited, integrate presence of Tannin.

Phenol

Ellagic test: The test solution was treated with few drops of 5% glacial acetic acid and 5% $NaNO_2$ solution. The solution turned muddy or niger brown precipitates occurred in the extract. It indicates the presence of phenols solution.

Phenol test: 0.5 ml of $FeCl_3$ solution was added into 2ml of test solution, formation of an intense bluish black colour indicates the presence of phenols.

To the extract add 5% of Potassium dichromate ($K_2Cr_2O_7$). Form Brown precipitates, indicates the presence of phenols.

Quantitative Phytochemical Analysis:

The powder each of leaf of all the 3 species was estimated for contents including flavonoids and total tannins. For the determination of each value three readings were taken. Details of methods used for each parameter have been described as follows:

Total flavonoids (Bohm and Kocipai, 1994)

10 gm of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No. 1. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Total tannins (Sadashivam and Manickam, 1969)

0.5 gm of the powdered material was transferred to a 250 ml conical flask. 75 ml of water was added to it. Flask was heated gently and boils for 30 min At 2,000 rpm it was centrifuged for 20 min and the supernatant was collected in 100 ml volumetric flask and volume was made up. 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml water. 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution was added and diluted to 100 ml with water. The absorbance was taken at 700 nm after 30 min. Water was used as a blank.

Folin-Denis Reagent: 100 gm sodium tungstate and 20 g phosphomolybdic acid was dissolved in 750 ml distilled water in a suitable flask and 50 ml phosphoric acid was added. The mixture was refluk for 2h and was making up to one liter with water. Protect the reagent from exposure to light.

Sodium Carbonate Solution: 350 gm sodium carbonate dissolved in one liter of water at 70- 80^{0}C. It was filtered through glass wool after allowing it to stand overnight.

Standard Tannic Acid Solution: 100 mg tannic acid was dissolved in 100ml of distilled water.

Working Standard Solution: 5 ml of the stock solution was diluted to 100ml with distilled water. One ml contains 50ugtannic acid.

Antioxidant activity (DPPH method)

The antioxidant properties were assessed by DPPH radical scavenging method (Padma *et al.*, 2006). The methanol extract was measured in terms of hydrogen donating or radical scavenging ability using a stable radical DPPH. 2.8 ml of DPPH solution (45µg/ml) were rapidly mixed with 200µl and 400µl of methanolic solution of plant extract one at a time in cuvette placed in the spectrophotometer. The absorbance at 515 nm was measured after 5 min. Radical scavenging activity or antioxidant property was evaluated as percentage was calculated as

$$\frac{(A0 - Atest)}{(A_0 - A_{ref})} \times 100$$

RESULTS AND DISCUSSION

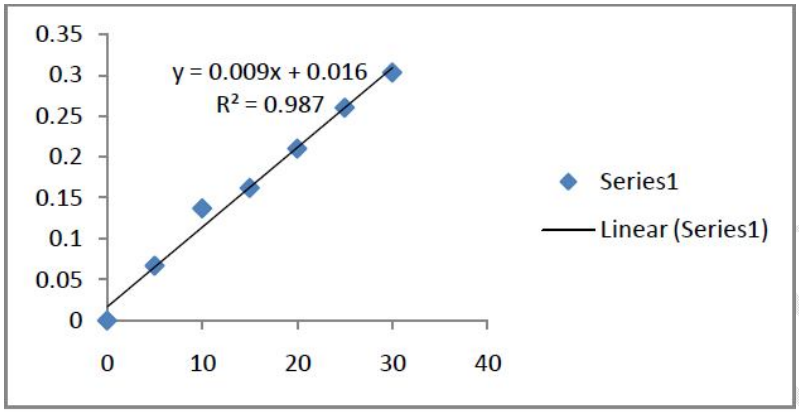
Table: 1. Qualitative assessment of phyto-constituents: (Microchemical tests)

Sr. No.	Reagent	Reaction	Inference	*B. roxburghiana* Viogt.			*B. hookeri* F.Muell.			*B. blakeana* Dunn.		
				Chl.	Met.	D.W.	Chl.	Met.	D.W.	Chl.	Met.	D.W.
1.	Extract + Iodine sol.	Blue colour	Starch	+	+	+	+	+	+	+	+	+
2.	Extract + Biuret reagent	Violet colour	Protein	-	-	-	-	-	-	-	-	-
3.	Extract + Molisch's reagent	Violet Ring	Carbohydrates	-	-	-	-	-	-	-	-	-
4.	Extract + Wagner's reagent	Reddish brown ppt.	Alkaloid	+	+	+	+	+	+	+	+	+
5.	Extract + dil. HCl + Mayer's reagent	Creamy colour	Alkaloid	-	-	-	-	-	-	-	-	-
6.	Extract + Hagars reagent	Yellow ppt.	Alkaloid	-	+	+	-	+	+	-	-	+
7.	Extract + 5% Lead Acetate	Yellow Ring	Flavonoid	+	-	-	+	-	-	+	-	-
8.	Extract + 10% $FeCl_3$	White ppt.	Flavonoid	-	+	+	-	+	-	-	+	-
9.	Extract + aq. NaoH and HCl Added	Yellow colour Disappeared	Flavonoid	-	-	+	-	-	-	-	-	+
10.	Extract + Zinc powder + Conc. HCl	Purple Colour	Flavonoid	-	-	-	-	-	-	-	-	-
11.	Extract + 1ml H_2O + aq. NaoH.	Yellow colour	Glycosides	+	-	-	+	-	-	+	-	-
12.	Extract + 2 drop of conc. H_2SO_4	Violet Ring	Glycosides	-	-	-	-	-	-	-	-	-
13.	Extract + Salkowaski reagent + 2ml Chl.+ 2 ml H_2SO_4	Green-yellow infloresence	Steroids	-	+	+	-	-	+	-	+	+
14.	Extract + 10% Lead Acetate	Yellow-Red ppt.	Tannins	+	+	+	+	+	+	+	+	+
15.	Extract + $K_2Cr_2O_7$	Brown ppt	Phenol	+	+	+	+	+	-	+	+	-
16.	Extract + 5% $FeCl_3$	Red ppt	Phenol	+	+	+	+	+	+	+	+	+
17.	Extract + 5% GAA + 5% $NaNO_2$	Muddy ppt	Phenol	-	+	+	-	-	-	-	-	+

In this chapter species specific results have been described. Powder leaves were subjected to chemical tests using standard reagents. Response obtained has been compiled in table form (Table 1).The data indicates presence of the secondary metabolites, alkaloids, flavonoids, glycosides, steroids and tannins in leaves.

Quantitative assessment of Phyto-constituents:

Powder leaves were subjected to quantitative assessment pertaining secondary metabolites and active ingredients. Results obtained have been compiled in Table No.2



Tannic acid Calibration Curve at 700nm

Table 2: Quantitative assessment of Phyto-constituents

Sr. No.	Content	*Bauhinia roxburghiana* Viogt.	*Bauhinia hookeri* F.Muell.	*Bauhinia blakeana* Dunn.
1	Flavonoids	47.5gm	14gm	5gm
2	Tannin	2.4gm	1gm	Traces

Tannin content was found maximum in *Bauhinia roxburghiana* Viogt. leaves, while it was found in traces in *Bauhinia blakeana* Dunn. The tannin and flavonoids content was present in the sequence *Bauhinia roxburghiana* Viogt> Bauhinia hookeri F. Muell. >*Bauhinia blakeana* Dunn.

Antioxidant property by DPPH method

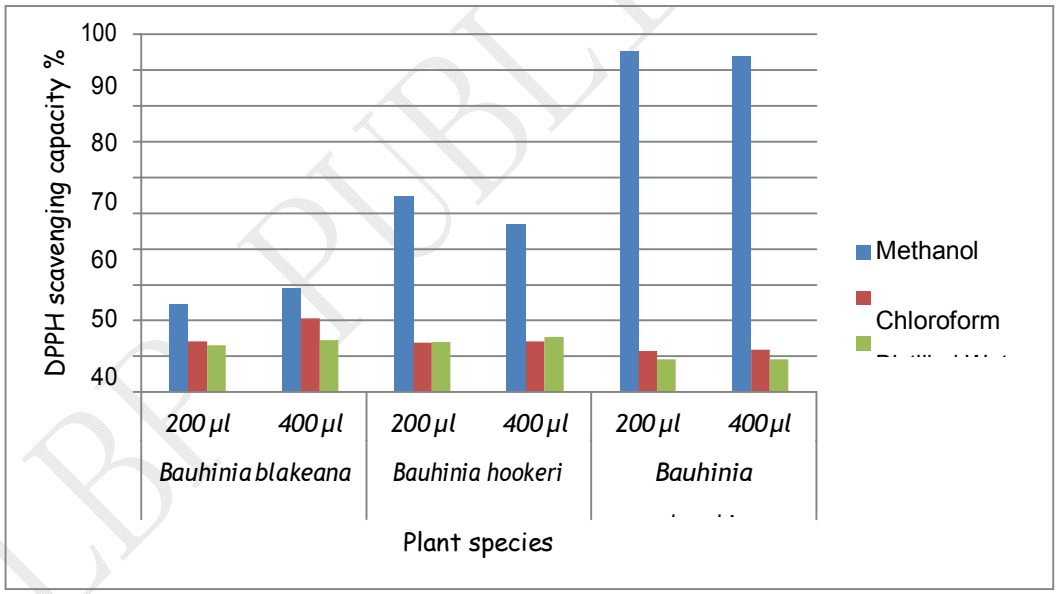
Ethanol extract was subjected to evaluate radical scavenging property using DPPH. Results obtained have been expressed in IC_{50} values as shown in Table 3. It seems from the data all the three plants showed potent antioxidant activity.

Table: 3 Antioxidant properties by DPPH method

Bauhinia species	Absorbance	DPPH Scavenging Capacity %

Bauhinia roxburghiana Viogt.	200 µl	400 µl	200 µl	400 µl
Methanol	0.014	0.148	95.15	93.71
Chloroform	2.085	2.075	11.51	11.81
Distilled Water	2.138	2.139	9.13	9.13
Bauhinia species	**Absorbance**		**DPPH Scavenging Capacity %**	
Bauhinia hookeri F.Muell.	200 µl	400 µl	200 µl	400 µl
Methanol	1.065	1.256	54.73	46.62
Chloroform	2.027	2.018	13.85	14.23
Distilled Water	2.024	1.992	13.98	15.34
Bauhinia species	**Absorbance**		**DPPH Scavenging Capacity %**	
Bauhinia blakeana Dunn.	200 µl	400 µl	200 µl	400 µl
Methanol	1.782	1.669	24.26	29.06
Chloroform	2.02	1.869	14.15	20.56
Distilled Water	2.043	2.011	13.17	14.53

Graph 1: DPPH Scavenging Capacity



Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well-known that plant produces these chemicals to protect them but recent research demonstrates that they can also protect humans and other living forms from diseases. Phytochemical studies have attracted the attention of plant scientists due to the development of innovative techniques. These techniques played a significant role in the search for additional resources of raw material for the pharmaceutical industry (Rajeswari, 2015)

Flavonoids are naturally occurring secondary metabolite in plants and are thought to have positive effects on human health. Studies on flavonoid derivatives have shown a wide range of antibacterial,

antiviral, anti-inflammatory, anticancer, and anti-allergic activities (Di Carlo *et al.,* 1999) (Montoro *et al.,* 2005) *Bauhinia roxburghiana* Viogt. Methanol extract show highest activity whiles the *Bauhinia blakeana* Dunn. has the lowest activity. Water extract has the lowest activity in all the species. Chloroform extract also shows the lowest activity except *Bauhinia blakeana* Dunn. Curative properties of medicinal plants are perhaps due to presence of secondary metabolites. Qualitative phytochemical characterization of leaves of three species of *Bauhinia* L. reveals presence of alkaloids in leaves; whereas presence of flavonoids, glycosides, saponins, steroids and tannins in leaves of all the species. Comparative data obtained have been compiled in table form (Table1). Presence of saponin, tannin, phenols, Flavonoids, steroids in leaves and the results obtained are quite comparable with these reports.

From data of quantitative determinations of secondary metabolites (Table: 2) it is found that two groups of phytochemicals viz. tannins, flavonoids are found in leaves but in less quantities *Bauhinia hookeri* F.Muell. and *Bauhinia blakeana* Dunn. these two species. Tannin contents also have been found maximum in leaves of *Bauhinia roxburghiana* Viogt. Considering contents of flavonoids, highest amounts are recorded *Bauhinia roxburghiana* Viogt. leaves and lowest in *Bauhinia blakeana* Dunn. Qualitative and quantitative estimations of phyto-constituents viz. proximate contents such as carbohydrates, proteins, starch and secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, steroids and tannins have been evaluated. Studies reveal presence of macronutrients -carbohydrates and proteins and secondary metabolites- tannins, flavonoids, alkaloids, and glycoside in leaves of all three species viz. *Bauhinia roxburghiana* Viogt., *Bauhinia hookeri* F.Muell. and *Bauhinia blakeana* Dunn. Plants are a source of a large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, and antimicrobials. A large number of the plants are claimed to possess antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of medicinal plants. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. Thus, *Bauhinia* species are easily available and has a promising therapeutic activity. This can be beneficially utilized for their medicinal properties to cure and solve various emerging diseases. This study showed the presence of antioxidant compounds flavonoids and tannins demonstrated some level of antioxidant activity. The total flavonoid content and the antioxidant activity of the leaves extract had significant correlation. Therefore, flavonoids are suggested to be a principal group of antioxidants in *Bauhinia.*

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