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Qualitative phytochemical analysis of various parts of bamboo (*Bambusa balcooa*) for possible therapeutic usages in bovine reproductive disorders

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Abstract
Biologically active compounds from natural sources have always been in great interest for scientists working on infectious diseases (Burkill, 2000; Roja *et al.*, 2000, Sofowora 1982 and Pemmal and Ignacimuthu, 2000). As a matter of fact, it has been estimated that today about 25% of all prescribed medicines are substances derived from plants (Egwaikhide and Gimba 2007; Zheng and Wang 2001). The phytochemical analysis of the plant is very important commercially and has great interest in pharmaceutical companies for the production of new drugs to be used in various diseases. Phytochemicals have two categories i.e. primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids. Secondary constituents contain flavonoids, alkaloids etc. The present study involves the phytochemical analysis of different parts of Bamboo (*Bambusa balcooa*) locally available in region of Uttarakhand. Bamboo is universally recognized for its various pharmaceutical properties. The aqueous, ethanolic and methanolic extract samples of leaves, stem and shoot were used for the analysis to find out the phytochemical constituents in the plant. These extracts were used for preliminary phytochemical analysis using standard chemical tests. Data indicates the presence of flavanoids, saponins, resins, fixed oils, phytosterols, phenols and tannins.

Keywords: Ethanolic, phytochemicals, bamboo, *Bambusa Balcooa*, flavonoids

Introduction
Use of medicinal plants for the treatment of various diseases has been part of human culture since ancient times. India has a rich resource of traditional herbal medicines to treat human and animal diseases. According to the All India coordinated project sponsored by the Ministry of Environment and Forest, New Delhi, 40% of 16,000 recorded flowering plants in India have ethnomedicinal value, whereas only 10% of these are used in drug and pharmaceutical industries. The intrinsic importance of these medicinal plants can very well prove as a potential source of new drug (Pushpangadan, 1997) [14]. A wide range of medicinal plants and their preparations are found to be useful in treatment of reproductive disorders and other related reproductive ailments (Nadkarni, 1954) [12]. Bamboos belonging to family Poaceae are considered as one of the most versatile multi utility forest tree grasses, also known in Indians as "Green Gold." It is one of the most valued medicinal plants that have been used for over 3000 years in Ayurveda and other traditional systems of medicine. They are known to have more than 1500 uses and are considered as one of the most economically important plants in the world (Lewington, 1990) [10].The beneficial therapeutic effect of bamboo species is seen in their continued use and benefits which are proven scientifically (Nazreen *et al.*, 2011) [13]. It has been used in ethno veterinary medicine for the treatment of various ailments in animals including retention of fetal membranes. Majority of the rural population in India was reported to utilize Bamboo plants for treatment of retention of placenta in animals. *Bambusa balcooa* also known as Female Bamboo is a tropical clumping bamboo originating from Northeast India. *Bambusa balcooa* is a clumping bamboo native to Indo-china and the Indian subcontinent (Roxb). It is thick walled clumping or sympodial bamboo. This bamboo species is often used as a food source, in scaffolding, for paper pulp or wood chips. The main constituents of bamboo culms are cellulose, hemicelluloses and lignin, which amount to over 90% of the total mass. The minor constituents of bamboo are resins, tannins, waxes and inorganic salts. Compared with wood, however, bamboo has higher alkaline extractives, ash and silica contents (Tomalang *et al.*, 1980) [21]. Bamboo contains other organic components in addition to cellulose and lignin. It contains about 26% starch, 2% deoxidized saccharide, 24% fat, and 0.86% protein (Rathod *et al.*, 2011) [16].

The pharmacological action of Bamboo plant has been shown the potential of anti-inflammatory effect, anti-microbial effect, immune-modulating effect, anti-stress, anti-oxidant effect which is believed to be due to phytochemical constituents. Phytochemicals are non-nutritive plant chemicals that have protective properties (Coffie *et al*., 2014) [5] Phytochemicals, also known as secondary metabolites or extractives, serve plants in their interactions with the animal world in the same manner as spines and thorns do. In most cases, the ecological function of phytochemicals is to protect plants from being fed on by herbivores (Berenbaum, 1999) [4]. In this work, we present the phytochemical screening of various parts of Bamboo plant to rule out its efficacy which can prove as a potential source of new drug.

Material and Methods

Collection of bamboo plant: Bamboo plant was collected from Agroforestry Research Centre, G.B. Pant University of Agriculture and Technology, Haldi. Bamboo plant was thoroughly cleaned to remove any debris. Leaves were dried in moisture free rooms whereas stem and shoot are cut into pieces and then dried and stored. After drying, various parts of bamboo were grinded separately in a grinder and powder was stored in a tight containers.

Preparation of Extract

Dried and powered form of Bamboo parts were soaked in aqueous, methanolic and ethanolic solutions for 72 hours. The solutions were filtered using muslin cloth, whatman filter paper No: 40 and then dried in rotary evaporator followed by final drying at 37°C. The dried extract was stored at 4°C until use.

Phytochemical analysis

All the extracts of Bamboo *Bambusa balcooa* were tested chemically for detection of various metabolites viz; alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, etc by using standard protocols (Shaik, 2011) [17].

Alkaloids

Dissolution of dried plant extract*:*

Extracts were dissolved individually in dilute hydrochloric acid and separated out by filteration. The filtrates were used to test for the presence of alkaloids by different tests as follows:

Mayer's test

The amount of 1.36 g of mercuric chloride was dissolved in 60 mL of distilled water and 5 g of Potassium iodide in 10 mL of water. The two solutions were mixed and diluted to 100 mL with distilled water. To 1 mL of acidic aqueous solution of extracts, a few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids.

Glycosides

Dissolution of dried plant extract:

The different extracts were hydrolyzed with 5 ml conc. HCl for 2 hours on a water bath and filter. The hydrolysate was subjected to the following tests.

Borntrage's test

In a test tube 2 ml of filtered hydrolysate were taken and into it added 3 ml of chloroform and mixed well. A chloroform layer was separated and to it 10% ammonia solution was added. A pink colour indicated the presence of glycosides.

Saponins

Froth test

In test tubes, 20 mg extract was suspended in 20 ml of distilled water and boiled for 5 min. 10 ml of the filtrate and 5 ml of distilled water was added and mixed well to develop the froth. The development of emulsion after mixing the froth with olive oil confirmed the existence of saponins.

Flavonoids

Shinoda Test

In a test tube 200mg of the extract was dissolved in 2 ml of methanol and heated. Few turnings of magnesium metal were added to the mixture followed by the addition of a few drops of concentrated hydrochloric acid. The appearance of an orange to red colour was indication of the presence of flavonoids.

Phenolic compounds

Lead acetate test

In a test tube 50 mg extract was dissolved in 5ml distilled water and added 3 ml of 10% lead acetate solution to this solution. A bulky white precipitate indicates the presence of phenolic compounds.

Proteins and amino acids

In a test tube 100 mg extract was dissolved in 10 ml of distilled water and filtered through whatmann no: 1 filter paper. The filtrate was subjected to tests for proteins and amino acids.

Ninhydrin test

In a test tube 2 ml of aqueous filtrate was taken and 2 drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added. A development of characteristics purple colour indicated the presence of amino acids.

Diterpenes

Copper acetate Test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of bright green colour indicated the presence of diterpenes.

Resins

Acetone-water Test:

In a test tube, extracts were taken and mixed with acetone the small amount of water was added and shaken. Appearance of turbidity indicated the presence of resins.

Phytosterols

Salkowski's Test

The different rhizome extracts were treated with chloroform and filtered. The filtrates were treated with 2-3 drops of Conc. Sulphuric acid, mixed carefully and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes.

Tannins

Ferric chloride test

In a test tube 2 mL of aqueous extract and 1-2 drops of 5% aqueous ferric chloride solution was added. A bluish black colour which disappears on addition of a few mL of sulphuric acid indicated the presence of tannins.

Estimation of total phenols and flavanols

The chemical assay of methanolic, ethanolic and aqueous of Bamboo plant were studied quantitatively by spectrophotometer in terms of total phenols and flavonoids and the concentration of these samples were measured with the help of standard calibration curve by the relation between absorbance and concentration of the sample.

Total phenolics assay

The total phenolics were determined by the Folin-Ciocalteu method developed by Singleton and Rossi, (1965) [19] and recently used by Chandra *et al*., 2016. [6] In brief, 1 ml of the sample extract was transferred into a test tube and mixed with 1 ml of 80% methanol and 8 ml of distilled water.

To each sample 0.5 ml of 1 N Folin-Ciocalteu reagent was added and mixed. After 5 min., 1 ml of saturated Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance of test sample was measured at 650 nm using a UV spectrophotometer (Thermo Scientific Evolution 201 series). The standard curve was drawn using various concentration of Gallic Acid and results were expressed as mg of Gallic Acid per gram of sample in dried weight (dw).

Total flavanols assay

Aluminium chloride colorimetric assay (Woisky and Salatino, 1998) [22] was applied for estimation of flavanols. 10 mg of extract were dissolved in 10 ml of 80% methanol to prepare stock solution. 0.1 ml of stock solution was mixed with 1.25 ml water and 0.75 ml of 5% $NaNO_2$ in a test tube. The mixture was incubated for 5 min. After incubation, 0.15 ml of 10% $AlCl_3$ was added to the mixtures. After 6 min. 0.5 ml of 1 N NaOH and 275 µL of distilled water was added, after proper mixing of the solution the intensity of pink colour was obtained at 510. The flavanol content standard curve was established using various concentration of catechin and the concentration were calculated with the help of calibration curve and expressed in mg / 100gm of dry material.

Result and discussion

The details of result for qualitative analysis of phytochemicals in water, methanolic and ethanolic crude extract of various parts of Bamboo plant are presented in (Tables 1, 2, and 3). Qualitative Phytochemical analyses of methanolic, ethanolic and aqueous extract of different parts of Bamboo were done for detection of various metabolites in which the leaves of Bamboo (*Bambusa Balcooa*) revealed the presence of flavanoids, saponins and phytosterols in all ethanolic, methanolic and aqueous extract whereas presence of resins and phenols were found only in aqueous extract and fixed oils were found to be present in methanolic and ethanolic extract. Similarly, Coffie *et al*., 2014 [5] performed the phytochemical analysis on three bamboo species (Bambusa vulgaris, Bambusa ventricosa and Oxytenanthera abyssinica) in which leaves of all the species contained saponins, general glycosides, coumarins and cyanogenic glycosides. Those of B. ventricosa and O. abyssinica contained polyphenols and flavonoids as well. However, there were no alkaloids, carotenoids, triterpenoids and steroids, anthraquinones and anthracene glycosides in any of the species/varieties. Bartholomew *et al*., 2013 [7] evaluated phytochemical properties of methanolic leaf extracts of the Nigerian Oxytenanthera *abyssinica* in which steroids (steroid glycoside), alkaloids, saponins, tannins, cardiac glycosides, flavonoids, anthroquinone and terpenes were detected while cyanogenic glycosides were absent. Similar findings were observed by Moses and Labunmi, 2015 [11] which showed that phytochemical screening of extracts revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, tannins, alkaloids in the leaf extracts of *Bambusa vulgaris.* Steroids and saponins were absent in the crude extracts. The plant extracts were good sources of different classes of bioactive compounds.

Table 1: Phytochemical analyses of methanolic, ethanolic and aqueous extract of *Bambusa balcooa* leaves:

Test	Methanolic extract	Ethanolic extract	Aqueous extract
Alkaloids	-	-	-
Flavanoids	+	+	+
Saponins	+	+	+
Glycosides	-	-	-
Resins	-	-	+
Fixed Oils	+	+	-
Phytosterols	+	+	+
Tannins	-	-	-
Phenols	-	-	+

Bamboo stem extract showed presence of saponins, resins, phytosterols and phenols in methanolic, ethanolic and aqueous extract whereas presence of flavanoids and fixed oils were seen in methanolic and ethanolic extract and presence of tannins was observed in aqueous extract.

Table 2: Phytochemical analyses of methanolic, ethanolic and aqueous extract of Bambusa balcooa stem:

Test	Methanolic Extract	Ethanolic extract	Aqueous extract
Alkaloids	-	-	-
Flavanoids	+	+	+
Saponins	+	+	+
Glycosides	-	-	-
Resins	-	-	+
Fixed Oils	+	+	-
Phytosterols	+	+	+
Tannins	-	-	-
Phenols	-	-	+

Bamboo shoot extract showed presence of flavanoids, resins, phytosterols, tannins and phenols in all three extracts whereas presence of saponins were observed in shoot extract showed presence of flavanoids, resins, phytosterols, tannins and phenols in all three extracts whereas presence of saponins were observed in ethanolic and methanolic extract. Singh *et al.*, 2012 [18] carried preliminary phytochemical screening of fermented Bambusa balcooa shoots and study revealed the presence of tannins, steroids, phenols, glycosides, flavanoids, carbohydrates and proteins. Ahmed, 2015 **[1]** detected the presence of a particular bioactive chemical in the methanolic and water extracts of Bamboo shoot. Natural products belonging to saponins, terpenoids, tannins, alkaloids, quinons and flavonoids were shown to be present in both the methanolic and aqueous extracts. However, phenol was only found in the aqueous extract. Baguistan *et al.*, 2017 **[12]** conducted screening of phytochemical constituents in ethanol and hot water shoot extracts of Bambusa vulgaris var. striata and Dendrocalamus asper in which cardiac glycosides, flavonoids, saponins and terpenoids were detected in both hot water and ethanol extracts of B. vulgaris var. striata and D. asper.

Table 3: Phytochemical analyses of methanolic, ethanolic and aqueous extract of Bambusa balcooa shoot:

Test	Methanolic extract	Ethanolic extract	Aqueous extract
Alkaloids	-	-	-
Flavanoids	+	+	+
Saponins	+	+	-
Glycosides	-	-	-
Resins	+	+	+
Fixed Oils	+	+	-
Phytosterols	+	+	+
Tannins	+	+	+
Phenols	+	+	+

The details of quantitative estimation of total phenols and total flavanoids are presented in Table 4 and 5. In the present study, the phenol content was measured by using three solvents. Ethanolic extract of bamboo leaves showed higher concentration of phenol as compared to methanol and water as solvent for extraction of phenols. In each extract total phenol content was higher in ethanol than in methanol and aqueous extract. Kaur *et al.,* 2015 **[9]** studied the total phenol content in which ethanolic extracts showed higher content of *B. arundinacea* followed by methanolic extracts and aqueous extract indicated that ethanolic extract has more antioxidant capacity than methanolic and aqueous extracts. Tongco *et al.*, 2013 [8] showed the total phenolic content in gallic acid equivalent (GAE) per 100 g dried sample of the Philippine bamboo "Buho" (Schizostachyum lumampao), was 76.72±9.06 for the ethanolic extract and 13.48± 4.12 for the aqueous extract. The more content was observed in alcoholic extract as compared to aqueous extract.

Table 4: Total phenolic content in ethanol, methanol and aqueous extract of various part of bamboo (*Bambusa balcooa*)

Total phenol (mg/g GAE)	
Ethanolic leaves	11.35±0.03
Ethanolic shoot	3.17±0.07
Ethanolic stem	2.09±0.02
Methanolic leaves	6.41±0.01
Methanolic shoot	2.65±0.01
Methanolic stem	1.41±0.02
Aqueous leaves	4.26±0.04
Aqueous shoot	1.89±0.03
Aqueous shoot	1.01±0.07

Total flavanoids

The study found that ethanol solvent is better in comparison to methanol and aqueous for extraction of flavonoid content. Parts of *B. Balcooa* was found to exhibit highest flavonoid content in ethanolic extract however aqueous extract show lowest flavonoid content. Bamboo has been widely known as one source of antioxidant. Studies on bamboo leaves have revealed flavonoid content in bamboo as antioxidant source (Sujarwo, 2010 and Qinxue, 2012) [20, 15].

Table 5: Total flavanoid content in ethanol, methanol and aqueous extract of various part of bamboo (*Bambusa balcooa*)

Total flavanoid (mg/g CNE)	
Ethanolic leaves	30.74±0.11
Ethanolic shoot	12.24±0.15
Ethanolic stem	2.26±0.04
Methanolic leaves	19.24±0.05
Methanolic shoot	8.91±0.09
Methanolic stem	1.99±0.04
Aqueous leaves	1.33±0.01
Aqueous shoot	0.88±0.01
Aqueous stem	1.45±0.01

Conclusion: It can be concluded from the present study that phytochemical analysis of various parts of *Bambusa balcooa* indicates the presence of flavanoids, saponins, resins, fixed oils, phytosterols, phenols and tannins. These herbal extracts can be used for curing diseases. Such active ingredients can be source of various drugs which could be used in different drug formulations for use in animals at lower price.

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