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Phytochemical, Antioxidant, Antimicrobial and Micropropagation Study of *Bambusa* Species

Nita Thapa[1], Janardan Lamichhane[1] & Dhurva P. Gauchan[1]
[1]*Department of Biotechnology, Kathmandu University, Dhulikhel, Kavre, Nepal*

Abstract:

Bamboo being a versatile plant provides numerous benefits to living beings comprising the environment. It is used in household works for construction, support; provides fodder for animals, edible shoots for human; helps environment by sequestering carbon *dioxide from the atmosphere and converting the* carbon *into plant fiber and this benefit continues. Besides, individual plant parts such as stem, leaves possess medicinal properties which is analyzed and evaluated through various studies of phytochemical, antioxidant and antimicrobial activity. Many people are benefitted economically by selling this plant so demand for its production is escalating day by day. Micropropagation is thus considered as crucial as this ensures production throughout the year irrespective of season and hindrance caused by flowering of this plant. Among many genus of this plant, Bambusa is considered as one of the important one and has many species under this genus. This review is thus an attempt to explore studies conducted by many scholars and researchers of this genus which is intended to provide light to some of the exceptional works done which eventually is beneficial to humankind.*

Keywords

Bambusa, Phytochemical, Antioxidant, Antimicrobial, Micropropagation

1. Introduction

Bamboos are perennial plants that belong to grass family Poaceae in the subfamily Bambusoideae. Woody bamboos consist of 9 to 10 sub tribes, containing between 59 and 111 genera and at least 1447 species [1]. It includes some of the fastest growing plants in the world due to its unique rhizome dependent system [2]. It does not require replanting after harvest as it will regenerate from its rhizome root structure. Besides, it improves soil quality and helps to rebuild eroded soil. The word bamboo is derived from the Kannada term 'bambu' which was found to be introduced through Indonesian and Malay to English [3]. It is likely that more than 1250 species under 75 genera of bamboo are unequally distributed in the humid tropical, subtropical and temperate regions of the world [4]. It is popularly known as "poor man's timber" since it is commonly used by rural people of many countries [5]. It serves as a great relief for rural people especially of Asian countries in economical as well as social terms. It is used as building material for construction purposes and people make money by selling bamboo as a whole, handicrafts and bamboo shoot for edible purposes and many more. Bamboo is also known as the "green gold of the forest" for of its global demand and versatile uses [6].
Bamboos are present with 75 genera growing in a wide range of different habitats worldwide so there is much variation in the characteristics and behavior they exhibit. *The Plant List* includes 509 scientific plant

names of species rank for the genus *Bambusa* out of which 130 are accepted species names. The Plant List includes a list of botanical names of plant species which was launched in 2010 and created by the Royal Botanic Gardens, Kew and the Missouri Botanical Garden. These accepted species are also included in World Checklist of Selected Plant Families (WSCP). *This list further* includes a further 97 scientific plant names of infraspecific rank for the genus *Bambusa* which is not included in WSCP. It is a genus of large clumping bamboos in the major group angiosperms (flowering plants). They are inherent to Southeast Asia, China, Taiwan, Himalayas, New Guinea, Melanesia and Northern Territory of Australia. They are also found to be acclimatized in other regions such as Africa, Latin America and different oceanic islands [7, 8]. Some members of the genus *Bambusa* species has been found to provide numerous benefits to science and communities [9, 10, 11]. Among twenty priority species of bamboo with economic importance which was listed in 'Priority Species of Bamboo and Rattan', compiled by the International Network for Bamboo and Rattan (INBAR), there are seven species of *Bambusa* genus [12]. INBAR released a revised list that included 20 taxa (species and genera) of particular economic importance and a further 18 taxa of importance [13]. Species of *Bambusa* genus are gaining more popularity nowadays because of various properties it possesses whether be it on construction work or better micropropagation capabilities. They also possess various antimicrobial capabilities and antioxidant activity because of different phytochemicals they inhibit. In this review paper, phytochemical assessment, antioxidant activity, micropropagation abilities and antimicrobial activity of various species of *Bambusa* genus is being reviewed.

2. Phytochemical Screening

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [14]. Plants are capable of synthesizing a wide variety of chemical compounds that perform important biological functions and these compounds are also used to defend against attack from predators such as insects, fungi and herbivorous mammals. Photochemical can be defined as the non-nutritive chemical compounds which occur naturally in plants where 'phyto' means plant in Greek. Extraction and characterization of several active phytocompounds from plants have given rise to some high activity profile drugs [15]. Many plant extracts and phytochemicals show antioxidant or free radical scavenging properties [16]. These phytochemicals are divided into primary and secondary metabolites. Primary metabolites are essential to all plants such as sugars, fats and secondary metabolites; also called phytochemicals are found in smaller range of plants and serve a specific purpose since these have protective properties.

There has been conduction of many investigations regarding study of phytochemicals on various medicinal plants from long time ago. However, study of these phytochemicals on bamboo leaves can be viewed as a novel practice and its interest is increasing more as each day passes. Bamboo leaves have therapeutic along with nutritive properties so it is a good source of fodder for livestock. Phytochemical

investigation was performed on three bamboo species *B. vulgaris* Schrad. ex J. C. Wendl., *B. ventricosa* McClure and *O. abyssinica* (A. Rich.) by Coffie et.al., 2014 in order to check its safety for consumption in both the dry and wet ethanol extracted leaf samples [17]. All the leaves extract was found to contain saponin, general glycoside, coumarin and cyanogenic glycoside and extract of *B. ventricosa* and *O. abyssinica* had polyphenol and flavonoid as well. There were no traces of alkaloid, carotenoid, triterpenoid, steroid, anthraquinone and anthracene glycoside in any of the species. *B. vulgaris* was regarded as the safest among these three species as it contained four classes of phytochemicals and contained relatively less saponin as these can lyse red blood cells of larger herbivores and this is why plant extracts are not directly injected into human beings [18]. Also their study concluded that the phytochemicals present in leaves extract were not considerably affected by air drying of the leaves because of which these leaves could be fed to livestock either in the fresh state or in the dry state as hay. In the dry season where there is low rainfall, lack of fodder for livestock is still a great problem in developing countries. Bamboos can be a better alternative for fodder as their leaves are carbohydrate rich when compared with components of the leaves from several vegetables [19]. Besides, these are nutritious, content of amino acid is high, has less microbial susceptibility and possess long shelf-life of their meal.

In the study performed by Joselin et.al, 2014, phytochemical analysis was done on the leaves extract of five different bamboo species *B. arundinacea* (Retz) Willd, *B. heterostachya* (Munro) Holttum, *B ventricosa* McClure, *B. vulgaris Schrad*. ex. J. C. Wendl. and *D strictus* (Roxb.) Nees [20]. Analysis was performed on six different solvents namely aqueous, petroleum ether, chloroform, ethanol and acetone along with Fourier Transform Infrared Spectroscopy (FTIR analysis). Aqueous solvent comparatively showed better presence of phytochemicals while quinones were completely absent in all the bamboo leaf extracts. Flavonoids and coumarins were found to be present in seven extracts whereas glycosides in six extracts indicating their way for future use as antioxidant agents. The bamboo leaf contains 2% to 5% flavone and phenolic compound that have the power to remove active free radicals, stop sub-nitrification and reduce blood fat [21]. The phytochemical screening of leaf extracts of *B. vulgaris* revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, tannins, alkaloids when extracted on hexane, chloroform, ethyl acetate and water solvents [22]. The plant extracts were good sources of different classes of bioactive compounds. These phytochemicals have therefore demonstrated good potential for the expansion of modern chemotherapies against microbial infections as the crude extracts considerably inhibited the growth of fungi *A. niger*.

Not only leaves and stem, bamboo shoots of few species are considered equally important since it has suitable health benefits because of their rich contents in proteins, carbohydrates, vitamins, fibres, minerals and very low fat. Bamboo shoots contain many minerals like potassium, calcium, manganese, zinc, chromium, copper, iron and also the minor amounts of phosphorus and selenium [23]. which are important for human body. Fresh bamboo shoot also possesses several vitamins such as Vitamin A, vitamin B1, vitamin B3, vitamin B6 [24]. Besides, bioactive compounds such as flavonoids, phenols and phenolic acids which are found to have potential health benefits are also found in bamboo shoot [25]. Its consumption as healthy diet is increasing rapidly mainly in

Southeast Asian and East Asian countries [26, 27]. The most widely distributed phytosterols, sitosterols are abundantly found in fermented bamboo shoots [28]. Fermented bamboo shoots have been eaten by various tribes since ancient times [29, 30]. Likewise, dish prepared with fermented bamboo shoots is taken as local delicacy in many ethnic communities. Phytochemical screening of fermented *B. balcooa* shoot revealed the presence of tannins, steroids, phenols, glycosides, flavanoids, carbohydrates and proteins when done in methanol and ethyl acetate solvents [31]. More phytochemical constituents were shown by methanol solvent in comparision to ethyl acetate. In study conducted by Srivastava, 1990, the concentration of total phytosterols in dried samples of fresh shoots of *B. tulda* and *D. giganteus* was 0.21-0.39% and 1.6-2.8% in samples of fermented shoots which was determined colorimetrically [32]. Anaerobic digestion was the cause for increase in level of phytosterols in fermented shoots as it degrades the organic matter. Therefore, fermented bamboo shoots could be used as a starting material for the production of steroidal drugs which can reduce inflammation and pain.

3. Antioxidant Activity

An antioxidant is broadly defined as any substance that delays or inhibits oxidative damage to a target molecule by trapping free radicals [33]. There is increment in the interest and study of antioxidant activity of various bamboo leaves among scholars and researchers worldwide although antioxidative compounds in bamboo leaves are not fully known. Many age related human diseases are also the result of cellular damage by free radicals and antioxidants could play a crucial role in preventing such diseases. The medicinal effects of bamboo leaves are mostly attributed by their antioxidant capacity [34]. When plant derived phytochemicals, are consumed in a regular basis, Halliwell B, 1996 pointed that it may drift the balance toward an ample antioxidant status [35]. The study of phytochemicals and potential antioxidant activity of plant extract is thus important in medical world as well. *Flavonoids* are diverse group of phytonutrients found in nearly all fruits and vegetables, grains, bark, roots, stems, flowers, tea and wine with more than 6,000 types. These are responsible for providing all those vibrant colors in fruits and vegetables along with carotenoids. These have the capacity to reduce free radical formation and scavenge them so interest is growing on study of relationship of flavonoids and antioxidant activity particularly for the reason of health benefits. Besides, the antioxidant capacities of many flavonoids are much found stronger than those of vitamins C and E [36]. Many groups of flavonoids have also been used in treatment of human diseases [37]. Both the antioxidant and prooxidant effects of flavonoids are discussed by Procházková et. al. 2011 in their review paper [38]. Flavonoids cannot only be purely accounted for antioxidants as meanwhile under certain reaction conditions they can also display prooxidant activity promoting the oxidation of other compounds. Nonetheless, prooxidant or antioxidant properties of a particular flavonoid depend most of all on its concentration. Flavonoids have also been stated to as 'nature's biological response modifiers as they possess inherent ability to alter the body's reaction to allergies, viruses and carcinogens [39].

Study of antioxidant activity of fifteen different bamboo species was performed by Wang et.al, 2007 among which eight species were of *Bambusa* [40]. Their results revealed that the methanolic extract of *B. textilis* McClure possessed the highest antioxidant activity among the selected fifteen bamboo species. It's

extract was then further analysed by HPLC-UV and HPLC-micro-fractionation to identify antioxidant compounds, where three antioxidant fractions namely isoorientin 4"-*O*-β-D-xylopyranoside, isoorientin 2"-*O*-α-L-rhamnoside and isoorientin were isolated. Evaluation of total polyphenol and antioxidant activity of leaves extract of *B. nutans* and *B. vulgaris* was carried out by Tripathi et.al, 2015 where total phenolic content of the extracts was calculated spectrophotometrically using a modified Folin-Ciocalteu method and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was used to assess antioxidant activity [41]. Their study revealed that *B. nutans* can be beneficial and has higher polyphenolic content and antioxidant activity than *B. vulgaris*. Therefore, it can be speculated that *B. nutans* can also be potentially useful as a natural source of antioxidant or in medicine. The higher antioxidant and superoxide scavenging activity in the chlorogenic acid (CGA) methyl ether compounds than in other phenolic acids and antioxidants is mediated through o-dihydroxyl and methoxyl properties of the molecules as well as inhibition of xanthine oxidase [42]. In their study, three CGA derivatives from the leaves of bamboo *P. edulis* was isolated and identified as 3- O-(3′-methylcaffeoyl)quinic acid, 5- O-caffeoyl-4-methoxylquinic acid and 3- O-caffeoyl-1- methoxylquinic acid. CGA is an ester of caffeic acid with quinic acid found naturally in various plants such as coffee beans, apples and blueberries. DPPH radical scavenging activity, total phenols and antioxidant activities was assessed and analyzed by Goyal et.al, 2010 in Indian wild *B. vulgaris* "Vittata" methanolic leaf extract [43]. There was found presence of carbohydrates, reducing sugars, flavonoids, steroids, saponins, alkaloids, tannins, anthraquinones and glycosides. The high contents of total phenolic compounds and total flavonoids indicated that these compounds contributed to the antioxidative activity. Phytochemical especially flavonoid is responsible for antioxidant activity and many species of *Bambusa* exhibit this property. Further research is still necessary for more qualitative and quantitative analysis of these compounds to derive potential use of bamboo leaves in medicinal world.

4. Antimicrobial Activity

In today's world, the discovery and development of antibiotics is considered as one of the most successful and powerful achievements of modern science and technology which is intended to control infectious diseases. There is increase in resistance of pathogens to traditional antimicrobial agents. This resistance has indicated in need for isolation of microbial agents which is less susceptible to regular antibiotics; also allowing the recovery of resistant isolates during antibacterial therapy. Although there is much advancement in medical world, there is still a critical need to control antimicrobial resistance by upgraded antibiotic usage in order to reduce hospital cross-infection [44]. The medicinal plants containing compounds with antimicrobial activity are found all around the world and many efforts have been made to discover novel effective antimicrobial compounds. Antimicrobial screening is done to specify the presence of antimicrobial molecule in the plant against pathogen and microbes.

Micro dilution assay showed that ethanolic extract of *D. asper* leaves extract was effective to inhibit all tested *E. coli* strains [45]. The chemical identification using pyrolysis GC/MS revealed that fatty acids, together with esters, long chain alcohols and aldehydes were the major compounds in ethanolic extract. Also, antidiarrheal agent could be extracted from *D. asper* leaves extract. In similar experiment, antibacterial

activity of *B. arundinaceae* was tested against the strains of *S. aureus, P.aeruginosa , E. coli* and *Bacillus* [46]. Ethanolic extract was moderately effective against all four organisms, while aqueous extract showed moderate effect against *E. coli* and *S. aureus*. Bamboo is considered as a good source of phytosterols that are the precursors of various pharmaceutically active steroids found in plants and these also act as nutraceuticals. In the study, conducted by Wasnik and Tumane, 2014, they found that the ethanolic and methanolic leaves extract of *B. bambose* L. showed excellent inhibitory action against all tested ten multiple drug resistant bacteria and they all were clinical isolates from wound infection [47]. Here also ethanol solvent comparatively showed better results like previous studies. Effectiveness of plant extract against microbial strains was determined by measuring zone of inhibition (ZOI) in all the studies. Ethanolic extracts of *B. arundinacea* and *M. indica* had higher inhibition on both gram positive as well as gram negative bacteria indicating both are potential source of natural antioxidants, phytochemicals and antibacterials. [48]. *M. indica* showed more therapeutic capacity. In this study also, ethanolic and methanolic extracts of both the tested plants were more effective than aqueous extracts. Antimicrobial screening of the n-hexane, chloroform and ethyl acetate extracts of *B. vulgaris* showed that the crude extracts inhibited the growth of fungi *A. niger*. This supports the ethno medicinal use of aqueous extracts of this species to treat sexually transmitted diseases and for wounds [22].

However, although bamboo leaves possess antibacterial activity there is not much studies and research conducted in order to find its efficacy and usefulness till date. Further investigations are still needed to identify their vital components needed for their effective role as therapeutic plant for those already studied extracts as well. Besides, additional work should also be carried out in order to fractionate the extracts to draw a better understanding of mechanism of those antioxidants responsible for antimicrobial activity.

5. Micropropagation

It is a rapid multiplication of a selected plant by use of vitro culture techniques. This technique utilizes modern plant tissue culture methods. Explants upon subsequent subcultures result in a number of multiple shoots and these multiple shoots on elongation allow rooting in vitro. After rooting, they are in vitro hardened and transferred to field [49]. Traditionally, bamboos were propagated with seeds, clump division, culm and rhizome cuttings but due to gregarious flowering, low seed viability, high costs, problems faced during long-distance transportation of vegetative propagules and poor efficiency of plant production alternative propagation methods were introduced [50]. Micropropagation of bamboo is the only viable method [51] and it has has allowed in developing new type of ornamental or commercial bamboo. The plantlet following this method can be produced in any time of the year without depletion in its quality. This method is regarded as the best available technique and will serve as standard for mass scale propagation of bamboo in the future [52]. For tissue culture of bamboo, the use of starting material i.e. seeds or adult plants including the choice of the propagation method are crucial [53]. In comparision to other medicinal plants, tissue culture in bamboo was practiced lately. The aseptic germination of bamboo seeds in *D. strictus* was stated by Alexander and Rao, 1968 [54] and this is reported to have indicated the start of tissue culture of bamboo in White, 1964 major and minor elements [55]. Following this, this medium has been then used by Nadguada et al, 1990 in *B. arundinacea* and *D. strictus*; Mascarenhas

et. al, 1988 in *D. strictus*; Ravikumar et. al., 1998 in *D. strictus* for zygotic embryo germination [56-58]. After use of this medium, Murashige and Skoog ,1962 (MS) basal medium which is more popular these days was regularly used in embryo germination of *B. arundinacea* where half strength of MS basal medium was used, in *B. nutans* and *D. membranaceus* and in *B. tulda.* MS medium is fortified with cytokinins, auxins, glucose source, gelling agent and carbon source according to the type of explant cultured as well as plant species involved [59-62]. Contamination and browning of explant is one of the main reasons of failure of tissue culture experiment. The severity of browning has varied according to species, tissue or organ, and nutrient medium and other tissue variables [63]. However, many techniques have already been evolved and studied to overcome these problems. Besides above mentioned, there are many other researchers who have accomplished successfully in propagating various bamboo species from zygotic embryo. Some of these are in *B. arundinacea* in Nitsch and Nitsch medium, 1967; in MS medium; in *B. vulgaris* in MS medium [64-67]. Various plant parts such as nodal segments, axillary buds were then used as explants as this ensured large scale propagation of bamboo. Micropropagation through axillary bud proliferation by nodal explants did not interfere with the production of true to type plant. This method also does not interrupt the callus phase and thus maintains the clonal fidelity [51]. This purpose of micropropagation was well satisfied by MS media so almost all researchers relied on using this medium as this media supplemented all the essential nutrients required for development of new plantlet invitro. Nodal explants were used by Arya et. al for *B. arundinacea* and *B. vulgaris*, Das and Pal and Islam and Rahman, for *B. balcooa*, Arya and Sharma for *B. bamboos*, Lin et.al for *B. edulis*, Shirin and Rana for *B. glaucescens*, Islam and Rahman and Yasodha et. al for *B. nutans*, Mishra et. al for *B. tulda*, Ndiaye et.al for *B. vulgaris* [68-76]. Likewise, axillary shoots was used as explant by for *B. atra* and axillary bud for *B. vulgaris* 'striata' [77-78].

Nodal explant is considered as the best starting material for micropropagation where multiple shoots are preferred or required as this is capable of providing such. An efficient micropropagation protocol for multiplication of *B. balcooa* from nodal explants was established where 88% success rate of acclimatization was achieved when done in liquid MS media [79]. In the same species, Patel et.al achieved survival rate of 74.66% when nodal segments were cultured on both solid and liquid MS media, Sharma and Sarma, achieved 100% success in field transfer, 90% success rate achieved by Mudoi and Borthakur whereas Ansari et. al achieved 90% success rate when culture was initiated in solid MS media [80-83] . In similar experiment, Raju and Roy established an efficient and reproducible micropropagation protocol for mass production and generation of uniform clones for *B. bambos* (L.) Voss where explant used was nodal segments containing unsprouted axillary buds in liquid MS media [84]. They recorded 100% survivability from well-established rooted plantlets during acclimatization process.

B. tulda is another species which researchers show great interest to mass propagate as its main purpose is on construction works and has high demand. An efficient and rapid method of in vitro propagation of this species from aseptic seedlings was studied and achieved 90% success rate upon field transfer when shoots from 3-week-old seedlings in liquid medium were used to initiate the cultures [85] whereas Mishra et.al had 98% rooting in liquid MS media [86]. In same species,

Waikhom and Louis had 81.81% success when culture was initiated on liquid MS media [87]. They take the privilege of successfully developing protocol which could produce rhizome in half-strength MS medium for the first time of this species. Survival rate was 100% when semisolid MS medium was used for culture of nodal explant [88]. *B. vulgaris* Schrad. ex Wendl has been promoted in order to solve the deforested environments and economic problems [89]. During their study they attained 75% survival where their explant was inter-nodal region of stem and culture medium was solid MS media. *B. nutans* is edible ornamental bamboo and is also propagated in vitro by many researchers for both study and commercial purpose. 95% survival rate was obtained by Mudoi et. al whereas 100% success rate was observed by Sharma and Sarma on field transfer when nodal explants were used in MS medium [90,91]. Research on other species of this genus is also being carried out which can ultimately contribute in mass production of bamboo.

6. Conclusion

There have been many developments in research techniques and equipment which has allowed for the assessment and evaluation of many constituents of plant molecule and same applies for bamboo plant also. It is undoubtedly a versatile plant and can serve to wellbeing of living beings in various ways. There has been conduction of lot of works to find the beneficial active constituents of some species, but still there is a long way to go to assess the true potential of all the species of this plant.

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