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Qualitative and Quantitative Phytochemical Analysis in leaf Extracts of Various Clones of *Neolamarckia cadamba* (Roxb.) Bosser

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Abstract

As sources of natural products, medicinal plants bear a great interest for researchers in recent decades and this interest has increased considerably in finding naturally occurring phytochemicals. *Neolamarckia cadamba* (Roxb.) Bosser is an important member of indigenous medicinal flora and used traditionally for the prevention of numerous diseases. The aim of the present work was focused to investigate and compare the phytochemical screening such as primary and secondary metabolites such as alkaloids, flavonoids, tannins, saponins, quinones, sterols, phenols, anthocyanin, proteins, carbohydrates, amino acid, terpenoids, steroids, glycosides, and quantification (total phenolics, tannin, and flavonoids) which were done. Phytochemical screening was done for selected 11 *N. cadamba* clones with different organic solvents (petroleum ether, ethanol, and hot water). The collected leaf samples were processed. Maximum number of nine secondary metabolites was obtained in the Clone No C.S, 13, 15, 42, 43, 60, 80, and 105. Rest of the clones have eight secondary metabolites. The maximum amount of total phenolic (200.7 mg Gallic Acid Equivalents [GAE]/g extract), tannin (196.26 mg GAE/g extract), and flavonoid contents (195.36 mg QE/g extract) were observed in Clone No 60. Hence, leaves of *N. cadamba* can be considered as a potential source of modern phytochemicals of medicinal importance for further studies.

Keywords: *Neolamarckia cadamba* and clones, Phytochemicals, Qualitative, Quantitative

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Introduction

Natural formulations as medication are becoming increasingly popular these days. In fact, when compared to chemical pharmaceuticals, numerous natural formulations that contain plant extracts have been determined to be safer treatments with fewer adverse effects. According to the World Health Organization (WHO), medicinal plants were the major source of healthcare for about 65% of the world's population.[1] Furthermore, it is believed that natural compounds, their derivatives, or analogues have accounted for nearly half of all medications created since 1980.[2] Further, it has been predicted that approximately 25% of the currently used modern medicines are derived from plants.[3]

Since ancient times, humans have used natural substances for their fundamental necessities such as food, clothing, housing, medicine, and other industrial reasons. It has been established that humans of all races utilized entire plants or particular parts of plants for medicinal purposes, with the oldest record dating back to 2600 BC in Mesopotamia for the usage of about 1000 plant-derived medicines.[4] Similarly, natural medicine molecules are documented in the Indian Ayurveda system, which is over 5000 years old. Around 2500 years ago, herbal medicine was also used in Chinese literature.[5] Recently, synthetic products are being restricted in the industries, due to harmful effects observed such as human toxicity and environmental pollution[6] and, besides, have been reported to be carcinogenic.[7] Furthermore, rising consumer expectations put pressure on industry, notably for safer goods.[8]

A growing trend toward the use of natural items rather than synthetic ones has been noted in increased demand for food, cosmetics, and pharmaceutical industries from this perspective. Plant natural products have been utilized in medicine, cosmetics, nutrition, and flavoring for thousands of years with no or little side effects. Thus, plant extracts appear to be a feasible alternative for this problem and the industries have put attention on the bioactive phytochemicals present in the plants. Phytochemicals found in plant extracts are generally non-toxic, effective at low doses, inexpensive, and environmentally benign. Furthermore, due to their antioxidant activity, new research has linked the consumption of vegetables, fruit, and herbs to the prevention of several bactericidal, antiviral, analgesic, anti-inflammatory, and anti-carcinogenic illnesses.[9]

Neolamarckia cadamba is locally called as vellai kadambu tree which has high economic importance. *N. cadamba* trees grow fast and are highly used as commercial wood species and used as medicine in treating various diseases such as dysentery, fever, and snake bites. Its bark, leaves, and flowers have been reported to have phytochemical compounds such as monoterpenoid, triterpenoid saponins, ethylene glycol, alkaloid, and cadambine, used in treatment of several diseases such as hyperglycemia, hyperlipidemia, hypertension, antihepatotoxic, antimalarial,

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anti-helminthic, antivenin, antitumor, and wound and skin diseases.[10]

Materials and Methods

Sample Collection and Botanical Identification of Collected Sample

The samples were collected in the Institute of Forest Genetics and Tree Breeding Research station. The collected leaf samples of *N. cadamba* clones were shade dried and powdered with a mechanical blender.

Extraction of Plant Material

The powdered leaf of each clone was packed in small thimbles separately and extracted successively with different solvents such as petroleum ether and ethanol in the increasing order of polarity using Soxhlet apparatus. Each time before extracting with the next solvent, the thimble was air dried. Finally, the sample was macerated using hot water with constant stirring for 24 h and the water extract was filtered. The different solvent extracts were concentrated by rotary vacuum evaporator (Yamato RE300, Japan) and then air dried. The dried extract obtained with each solvent was weighed. The percentage yield was calculated in terms of air-dried weight of extract from each solvent. The stock solution of the extract obtained was prepared (1 mg/mL of respective organic solvents) and used for further analysis.

Qualitative Phytochemical Screening

The leaf extract of *N. cadamba* clones were analyzed for the presence of major phytochemicals such as carbohydrates, proteins, amino acids, alkaloids, tannins, saponins, phenolic compounds, glycosides, flavanol glycosides, cardiac glycosides, phytosterols, and gums and mucilage according to standard methods.[11]

Quantification Assays

Total phenolics

The total phenolics of the leaf extracts were determined according to the method described.[12] The results were expressed as gallic acid equivalents (GAEs).

Tannins

The total phenolics contain both tannin and non-tannin phenolics. The amount of tannins was calculated by subtracting the non-tannin phenolics from total phenolics. The method described[12] was used for determination of non-tannin phenolics. The analyses were also performed in triplicates and the results were expressed in tannic acid equivalents.

Flavonoids

The flavonoid contents of all the four extracts were quantified according to the method described.[13] Rutin was used as the standard for the quantification of flavonoids. The experiment was done in triplicate and the results are expressed as Rutin equivalents.

Results And Discussion

Qualitative Phytochemical Screening

The qualitative phytochemical screening in leaf of *N. cadamba* clones was tested for major primary and secondary phytochemicals the results are shown in Tables 1–3. The results revealed that the primary metabolites such as carbohydrates, proteins, and amino acids were present in all the clones.

The secondary metabolites such as alkaloid, tannin, phenolic compounds, flavanol glycosides, cardiac glycosides, and phytosterols are present in all the extract. The +sign indicates a high concentration of particular secondary metabolites which were indicated by the high intensity of the color developed and sign indicates the absence of chemical compounds. However, saponins, glycosides, and gums and mucilage's were absent in all the extracts. Phytochemical compounds are medicinally valuable compounds which are synthesized by plants themselves for protection. Alkaloids are powerful poisonous substances which have high biological activity such as anti-inflammatory[14] antimalarial,[15] antimicrobial,[16] and cytotoxicity, antispasmodic, and pharmacological effects.[17]

Quantification of Secondary Metabolites in Leaves of *N. cadamba* Clones

Quantification of Total Phenolics Content

The amount of total phenolics presents in leaf extracts of different clones is analyzed and shown in Figure 1. The total phenolics were found to be higher in ethanol extracts of leaf in clones C. No 60 (200.7 mg GAE/g extract) followed by C. No. 15 (187.57 mg GAE/g extract), C. No. 17 (180 mg GAE/g extract), C. No. 43 (174.94 mg GAE/g extract), and C.s (174.44 mg GAE/g extract).

The multiple hydroxyl groups in the chemical structure of polyphenols make them ideal for free radical-scavenging reactions and as metal chelating agents.[18] The arrangement of the hydroxyl groups around the phenolic molecule is also important for antioxidant reactions.[19] The higher amount of phenolics obtained by the solvents like ethanol could be due to higher solubility of phenolics and other aroma compounds. It has been already investigated in many plant species that the total phenolics could significantly contribute to the antioxidant capacity of that species. Therefore, the higher amount of phenolics present in *N. cadamba* can be taken as a good indication for its higher antioxidant capacity.

Quantification of total tannin content

The amount of total tannin present in leaf extracts of different clones is analyzed and shown in Figure 2 which showed the amount of tannins present in ethanol leaf extracts of *N. cadamba* clones. The total tannin was found to be higher in C. No 60 (196.26 mg GAE/g extract) followed by C. No. 15 (177.05 mg GAE/g extract), C. No. 17 (174.68mg GAE/g extract), C. No. 43 (170.87 mg GAE/g extract), and Cs (173.37 mg GAE/g extract).

The greater amount of tannins in the extracts of the stem of *Anthocephalus indicus* can be due to higher polymerization of

Table 1: Preliminary phytochemical analysis of *Neolamarckia cadamba* leaf of clones (Petroleum ether extracts)

Phytochemicals	*C.NO C.S*	*C.NO 13*	*C.NO 15*	*C.NO 17*	*C.NO 22*	*C.NO 42*	*C.NO 43*	*C.NO 60*	*C.NO 67*	*C.NO 80*	*C.NO 105*
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+
Amino acid	+	+	+	+	-	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-
Phenolic compounds	+	+	+	+	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-	-	-	-
Flavanol glycoside	+	+	+	+	+	+	+	+	-	+	+
Cardiac glycoside	+	+	+	-	+	+	+	+	+	+	+
Phytosterols	+	+	+	+	+	+	+	+	+	+	+
Gums and mucilage	-	-	-	-	-	-	-	-	-	-	-
Total compounds	9	9	9	8	8	9	9	9	8	9	9

Present (+); Absent (-): C. NO - Clone Number

Table 2: Preliminary phytochemical analysis of *Neolamarckia cadamba* leaf of clones (Ethanol extracts)

Phytochemicals	*C.NO C.S*	*C.NO 13*	*C.NO 15*	*C.NO 17*	*C.NO 22*	*C.NO 42*	*C.NO 43*	*C.NO 60*	*C.NO 67*	*C.NO 80*	*C.NO 105*
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+
Amino acid	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-
Phenolic compounds	+	+	+	+	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-	-	-	-
Flavanol glycosides	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	+	+
Phytosterols	+	+	+	+	+	+	+	+	+	+	+
Gums & Mucilage	-	-	-	-	-	-	-	-	-	-	-
Total Compounds	9	9	9	9	9	9	9	9	9	9	9

Present (+): Absent (-): C.NO - Clone Number

Table 3: Preliminary phytochemical analysis of *Neolamarckia cadamba* leaf of clones (Aqueous extracts)

Phytochemicals	*C.NO C.S*	*C.NO 13*	*C.NO 15*	*C.NO 17*	*C.NO 22*	*C.NO 42*	*C.NO 43*	*C.NO 60*	*C.NO 67*	*C.NO 80*	*C.NO 105*
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+
Amino acid	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	-	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-
Phenolic compounds	+	+	+	+	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-	-	-	-
Flavanol glycosides	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	-	+	+	+	-	+	+
Phytosterols	+	+	+	+	+	+	+	+	+	+	+
Gums & Mucilage	-	-	-	-	-	-	-	-	-	-	-
Total Compounds	9	9	9	9	7	9	9	9	8	9	9

Present (+): Absent (-): C.NO - Clone Number

existing polyphenolic compounds. Recently, it has been reported that the high molecular weight phenolics such as tannins have more ability to quench/scavenge free radicals. Tannins are constituents of several drugs due to their astringent property. They are used in the treatment of hemorrhoids, diarrhea, dysentery, leucorrhea, and as a useful medicine for the throat.[20] Since the tannin content was low in leaf extracts, there will not be any unfavorable reaction caused by tannin as anti-nutritional factors. Moreover, the tannin content in leaf of *N. cadamba* clones may enhance the free radical scavenging activity of its extracts.

Quantification of Flavonoid Content

The flavonoid contents were analyzed in the *N. cadamba* leaf of clones and the results are shown in Figure 3. The total flavonoid was found to be higher in ethanol extracts of C. No 60 (195.36mg QE/g extract) followed by C. No. 17 (180.63 mg QE/g extract), C. No. 42(173.96 mg QE/g extract), C. No. 15 (172.91 mg QE/g extract), and Cs (163.78 mg QE/g extract).

Flavonoids, which include flavanols, isoflavanols, and chalcones, are one of the most varied and significant classes of natural phenolics. Due to their ability to scavenge free radicals and

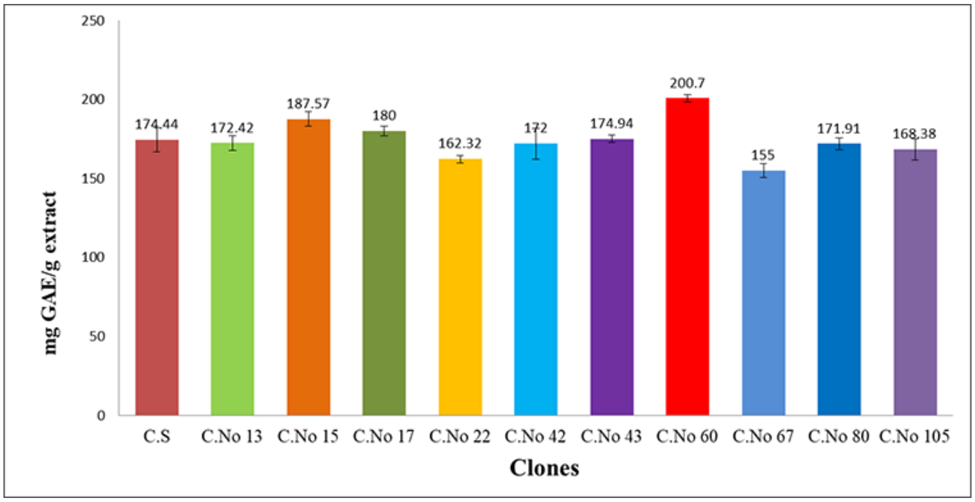

Figure 1: Total Phenolic Contents of *Neolamarckia cadamba* leaf of clones. GAE: Gallic acid equivalents, values are mean of triplicate determination (*n*=3) ± standard deviation

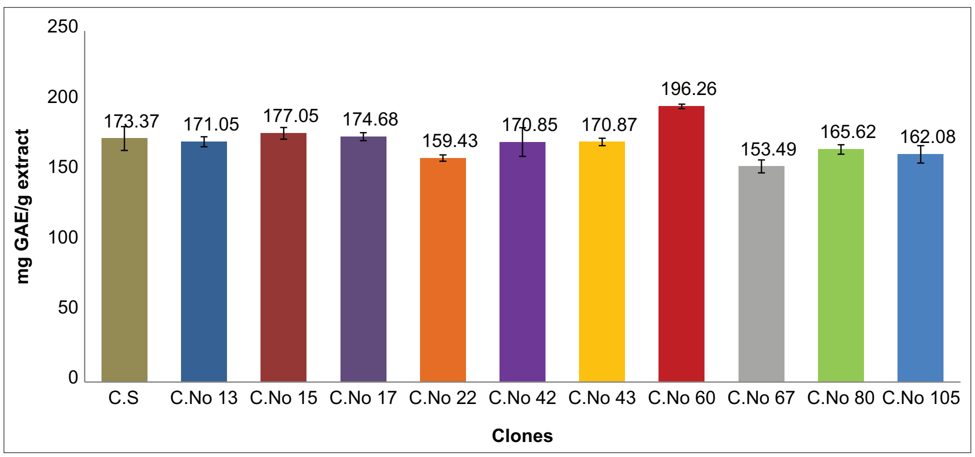

Figure 2: Total tannin content of *Neolamarckia cadamba* leaf of clones. GAE: Gallic acid equivalents, values are mean of triplicate determination (n=3) ± standard deviation

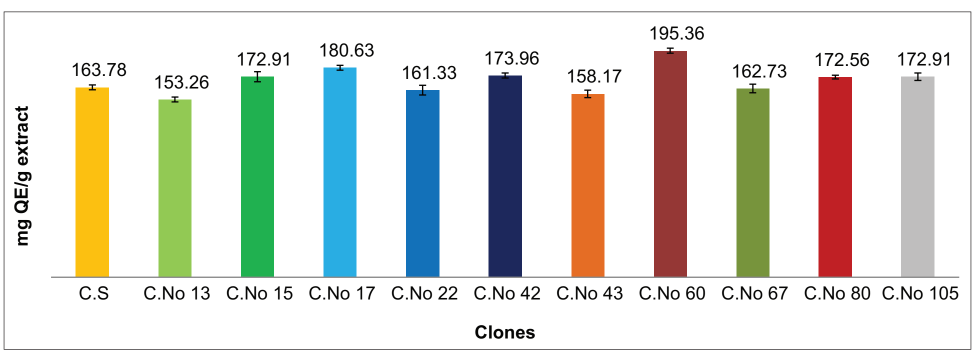

Figure 3: Total flavonoid content of *Neolamarckia cadamba* leaf of clones. QE: Quercetin equivalents, values are mean of triplicate determination (*n*=3) ± standard deviation

active oxygen species, polyphenolic substances like flavonoids have been characterized as high levels of natural antioxidants. Flavonoids have been linked to anticancer, anti-diabetic, anti-aging, and cardiovascular disease preventative activities.[21] Quantification of flavonoid concentration in plant parts is significant since it may be linked to the plant's anticancer and radical-scavenging action. Since *N. cadamba* possess good flavonoid content in the leaf, it could be assumed that it can have a higher free radical scavenging activity which involves the transfer of electron or hydrogen atom from flavonoids to free radicals.

Conclusion

As a consequence, the findings of this investigation clearly demonstrated that the leaf of *N. cadamba* clones is more effective. More research is required to isolate and identify specific bioactive chemicals, as well as *in vivo* investigations to understand their mechanism of action as an antioxidant medication that might be a cost-effective and dependable source of medicine for human health.

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