

Evaluation of phenolic compounds from different parts of *Neolamarckia cadamba* (Roxb.)

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ABSTRACT

The present study was conducted to evaluate the antioxidant property depending on phenolic compounds content of *Neolamarckia cadamba* (Roxb.) leaves, stem and fruit. Phenolic compounds are vital in defense responses including anti-inflammatory, anti-aging, antioxidant and antiproliferative activities also involve in defiance against UV radiation. Antioxidants have been reported to prevent oxidative damage caused by free radicals and can be used in the treatment of cardiovascular diseases. The main objective of this study is to evaluate the total phenol, ortho dihydric phenol, bound phenol and tannins. For determination of phenolic compounds, 1g leaves, stem and fruit were used. The highest amount of total phenol, ortho dihydric phenol, bound phenol was determined from the leaves while the least amount was determined from the fruit of *Neolamarckia cadamba* (Roxb.).

Key words: *Neolamarckia cadamba* (Roxb.), Phenolic compounds, Spectrophotometer, Antioxidant.

INTRODUCTION

Medicinal plants hold significant value in both modern and traditional medicine due to their diverse therapeutic properties. Their medicinal benefits stem from the presence of various phytoconstituents, including flavonoids, alkaloids, tannins, and phenolic compounds, which are considered pharmacologically active. These compounds play a crucial role in human health, primarily due to their antioxidant properties. They function as antioxidants, structural polymers (such as lignin), attractants (like flavonoids), signal molecules (such as salicylic acid and flavonoids), and defense agents (including tannins and phytoalexins). Phenolic compounds derived from plants may aid in managing carbohydrate metabolism disorders, such as diabetes, by inhibiting amylase absorption. Additionally, plants produce these phenolic compounds as a defense mechanism against pathogens, parasites, and predators. Their synthesis is also influenced by environmental and physiological factors, particularly when the plant is subjected to stressors such as pathogen attacks, insect predation, UV radiation exposure, or physical damage. (Chung *et al.*, 2003; Crozier *et al.*, 2006; Diaz Napal *et al.*, 2010; Kennedy and Wightman, 2011). In biological systems, cells can suffer DNA damage, as well as lipid and protein oxidation, due to the presence of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Excessive production of these reactive species can be triggered by external factors such as cigarette smoke, radiation, alcohol consumption, and environmental pollutants. The human body naturally counteracts these harmful radicals through its intrinsic antioxidant system, which helps maintain a balance between oxidation and antioxidation. However, supplementing with external antioxidants can mitigate oxidative stress by preventing the initiation or progression of oxidative chain reactions. These antioxidants function as free radical scavengers, singlet oxygen quenchers, and reducing agents, thereby protecting cellular components from oxidative damage. (Baiano and del Nobile; 2016). The Cadamba tree, a significant medicinal plant from the Rubiaceae family, is known for its abundance of phytochemicals and secondary metabolites, including cadambagenic acid, cadamine, quinovic acid, β -sitosterol, and cadambine, which exhibit various pharmacological and biological activities. It serves as a natural alternative to synthetic chemical compounds for preventing and treating multiple chronic diseases. Over a century of research has been dedicated to identifying its bioactive compounds and their applications. However, only a small fraction ($\leq 2\%$) has been commercialized due to the absence of an appropriate model system and associated controversies. Additionally, the solubility of these phytochemicals presents challenges, as the impact of different solvents used for extraction remains unpredictable. Beyond its medicinal value, Cadamba is also an ornamental tree with religious significance.

Neolamarckia cadamba (Roxb.) is a medicinal plant known for its rich phytochemical composition, including flavonoids, alkaloids, tannins, and phenolic compounds, which contribute to immune enhancement. Its antioxidant properties help neutralize free radicals, reducing oxidative stress that weakens immunity. The plant exhibits antimicrobial and anti-inflammatory effects, supporting the body's defense mechanisms against infections. Additionally, its bioactive compounds aid in modulating the immune response, promoting overall health. Regular consumption of extracts from *N. cadamba* may strengthen immunity by enhancing cellular defense, reducing inflammation, and supporting detoxification. Further research is needed to explore its full potential as a natural immunomodulator.

MATERIAL AND METHODS:

Plant material

The extensive survey, identification and collection of plant from Amravati region was carried out. Plant identification was carried out with the help of floras (Cook, 1957; Dhore, 1986; 1998; Naik, 1998).

Preparation of plant material

Fresh leaf, stem and fruit were collected, dried under shade, finely powdered and stored in airtight container. All plant parts were powdered separately. 1gm each of each plant part was taken for estimation of phenolic compounds gm/ μ gm.

METHODS:

Estimation of Total phenol, Bound phenols, Tannin and ortho-dihydric phenol were done according to the methods prescribed by Thimmaiah (1999), which are given below.

Estimation of total Phenols

1gm of sample was grind with the help of mortar and pestle with 10 ml of 80% ethanol. And centrifuged at 10,000 rpm (20minutes). Supernatant was collected and evaporated to dryness after dryness residue was taken into a test tube and makeup the volume with 5ml distilled water. 1ml aliquot was Pipette out in test tube, and make up the volume up to 3ml with distilled water. 0.5ml of Folin- Ciocalteu reagent was added. After 3 minutes, into each tube 2 ml of 20% Na_2CO_3 solution was added. Mixed thoroughly and tubes was kept in boiling water for 1minute, then allowed to cool and absorbance was measured at 650nm against reagent blank. Reagent blank was prepared similarly without the extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1ml) of catechol. (Thimmaiah S.R. 1999).

Estimation of Bound Phenols

1gm sample was grind with the help of mortar and pestle with 5ml of SDS solution and centrifuge for 5 min and supernatant was discarded. The residue was wash with once 5ml SDS solution, twice with 5ml of water, twice with 5ml of ethanol and twice with 5ml of diethyl ether (after each washing centrifuge and the supernatant was discarded). Allow the residue to dry and was suspended in 3ml of 0.5 M NaOH. It was kept overnight at room temperature. In the next morning it was Centrifuge and the supernatant was diluted 1:10 with 0.5 M NaOH (0.5 ml supernatant and 5 ml of 0.5 M NaOH). Absorbance was measured at 290 nm against a reagent blank lacking only extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol. (Thimmaiah S. R. 1999)

Estimation of Ortho- dihydric phenols:

1gm sample was grind with the help of mortar pestle with 10ml of 80% ethanol. And centrifuged it at 10,000 rpm (20 minutes) and supernatant was collected and evaporated to dryness. After drying residue was taken and volume made up to 5ml with distilled water. 1 ml of aliquot was pipette out in a test tube, in this test tube 1ml

of 0.05 N HCL, 1 ml of Arnov's reagent, and 10 ml of distilled water and 2ml of 1N NaOH was added. Absorbance was measured at 515 nm against a reagent blank lacking only extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol. (Thimmaiah S. R. 1999).

Estimation of Tannins: Vanillin hydrochloride method was used.

1 gm of sample was mixed in 10ml methanol after 20-28 hrs. Centrifuged and supernatant was collected. 1ml of supernatant was pipette out into test tube and quickly added 5ml of vanillin hydrochloride reagent and mixed. After 20 min absorbance was read at 500nm. A reagent blank was prepared with vanillin hydrochloride reagent alone. A catechins standard graph was prepared from working standard (100 μ g/ml) of catechins and amount of tannins was calculated. (Thimmaiah S. R. 1999). A standard graph was obtained by plotting concentration on X-axis and the corresponding values of absorbance along Y- axis on a graph paper resulting straight line which passes through the origin and maximum points of standard reading. It is used to quantify the amount of a given compound present in an unknown sample whose absorbance value is matched against that of standard along-axis and a corresponding concentration could be read off along-axis. (Thimmaiah S.R. 1999).

RESULT AND DISCUSSION

Sr. No	Phenolic compounds	Wt. of sample	Total volume of extract	Volume taken for analysis	μ gm/gm		
					Leaf	Stem	Fruit
1	Total phenol	1 g	10 ml	1 ml	650	450	210
2	Tannins	1 g	5 ml	1 ml	650	1050	40
3	Bound Phenols	100 mg	5 ml	1 ml	8250	6150	3600
4	Ortho-dihydric phenol	1 g	10 ml	0.5 ml	4450	3400	1950

The results showed that the maximum amount of Total Phenol (650 μ gm/gm), Bound Phenols (8250 μ gm/gm) and ortho-dihydric phenol (4450 μ gm/gm) was observed in leaf of *Neolamarckia cadamba*. While the least amount of Total Phenol (210 μ gm/gm), Bound Phenols (3600 μ gm/gm), Tannins (40 μ gm/gm) and ortho-dihydric phenol (3600 μ gm/gm) was observed in fruit of *Neolamarckia cadamba* (Roxb.). Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. The nitric oxide scavenging activity of flavonoids and phenolic compounds are known (KimH.*et al.*, 2002). Phenols are present in food; they may have an impact on health and most are known to have an antioxidant activity. (Demitrios2006).

Many studies have been reported that coronary heart disease and cancer mortality reduced when dietary intake of natural phenolics increased, it also found effective in various health-related properties like anticancer, anti-inflammatory, antioxidant and antiviral activities (Ghafar, *et al.*, 2010). Phenolic compounds especially flavonoids, phenolic acids and tannins, act as inhibitor of α glucosidase and α -amylase, which are responsible for the digestion of dietary carbohydrates to glucose. (Lin, *et al* (2016). tannins show the antimicrobial activity, tannins of unknown origin inhibited the growth of filamentous fungi *Fomes annosus* with a minimum inhibitory concentration (MIC) greater than 0.5 g/l (Haars *et al.*1981). Eyong, *et al* (2008) investigated that quinones exhibit antibacterial, antioxidant, ant plasmodial, neurological, antitumor, trypanocidal and anti-HIV activity.

Research on medicinal plants has increased over time in an effort to understand their mode of action and support the claims made by traditional healers. One aspect of this study has been the investigation of A.

marmelos's bioactive elements and antioxidant capabilities. The results of this study have confirmed that restorative plants may be a useful source of antioxidants. It is known that flavonoids have a major antioxidant effect on the fitness and health of humans. Flavonoids function by means of chelating or scavenging mechanisms (Kessler *et al.*, 2003; Cook and Samman, 1996 [11, 9]. Because of its great capacity to scavenge free radicals, phenolics may be because of their abundance of phenolic hydroxyl groups (Sawa *et al.*, 1999)

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, flavonoids, carbohydrates steroids. (Edoga *et al.*, 2005; Mann1978).

CONCLUSION:

This study highlights that the leaves of *Neolamarckia cadamba* (Roxb.) are rich in total phenols, ortho-dihydric phenols, tannins and bound phenol, supporting its traditional medicinal use. The plant exhibits strong antioxidant properties and possesses a wide range of therapeutic benefits, including anti-aging, anti-inflammatory, anticancer, antidiabetic, antimicrobial, antiplasmodial, neurological, antitumor, and anti-HIV activities, while also playing a role in immune modulation. Despite being a common weed, *Neolamarckia cadamba* (Roxb.) remains underexplored. These findings provide valuable insights for the pharmaceutical industry, promoting its potential applications in human health. The growing preference for natural antioxidants further reinforces the importance of this plant as a safer alternative to synthetic drugs.

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