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Original Research Article

Phytochemical Screening and Antioxidant Activity of *Neolamarckia cadamba* and *Cymbopogon citrates* from Durg District of Chhattisgarh, India

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Abstract

Medicinal plants have attracted a great deal of scientific interest due to their potential as a source of natural biologically active compounds especially the antioxidant properties of medicinal plants that have created its wider applications including pharmaceuticals, alternate medicine, and natural therapies. The present study was to evaluate the qualitative estimation of phytochemicals and antioxidant properties from leaves, stem, and root of *Neolamarckia cadamba* and *Cymbopogon citratus*. In our study, we found the presence of steroids, flavonoids, alkaloids, tannins, saponins, and cardiac glycosides and antioxidant features in both plants and found suitable for the therapy of oxidative damages. In the present study, free radical scavenging percentage activity was found maximum in aqueous stem extract of *Neolamarckia cadamba* (81% with 1.5 ml extract) and least in aqueous stem and leaf extract of *Cymbopogon citrates* (1.67% and 1.67% with 0.5 ml respectively). From the present work, it becomes possible to conclude that these plants could be a promising source of bioactive compounds and warrant further study.

Keywords: Antioxidant, Pharmaceuticals, *Neolamarckia cadamba*, *Cymbopogon citratus*.

INTRODUCTION

Several antioxidant secondary metabolites have been reported from fruits and vegetables including phenolics, carotenoids, anthocyanins, and tocopherols [1]. Approximately 20% of known plants have been used in pharmaceutical studies, impacting the healthcare system in positive ways such as treating cancer and harmful diseases [2]. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables [3]. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants [4].

Various studies have shown that many plants are a rich source of antioxidants, for instance, vitamins A, E, and phenolic compounds such as flavonoids, and tannins, found in plants, act as antioxidants [3]. Antioxidants reduce the oxidative damage in foods by delaying or inhibiting oxidation caused by reactive oxygen species (ROS), ultimately increasing the shelf-life and quality of these foods [5]. Beta carotene, ascorbic acid, and many phenolics play dynamic roles in delaying aging, reducing inflammation, and preventing certain cancers [6].

Neolamarckia cadamba (family Rubiaceae) is an evergreen, tropical tree native to south and Southeast Asia. It has scented orange flowers in dense globe-shaped clusters. The tree is grown as an ornamental plant and is used for timber and paper making. Its height is up to 48 m and leaves are 13-32 cm long. It is a medicinal plant used to cure several diseases and ailments such as Diabetes, Cardiovascular Disorder, Cancer, and Liver damage [7]. The reported uses of this plant are anti-hepatotoxic, ant-malarial, anti-microbial, wound healing, antioxidant, anthelmintic, analgesic, anti-inflammatory, antipyretic, diuretic and laxative, and for antitumor activity. The decoction of the bark of the plant is used for gargling to treat mouth ulcers and inflammations of the gums. The juice of the fruit of the plant is used in a dose of 40-50ml to treat excessive sweating, thirst, and burning sensation of the body [8].

Cymbopogon citratus (family Poaceae) is a tropical plant from South Asia and introduced to South East Asia. The dried leaves can also be brewed into tea, either alone or as a flavoring in other teas, imparting a

flavor reminiscent of lemon juice but with a mild sweetness without significant sourness or tartness. It is used as an herbal medicine for a wide range of applications based on its antibacterial [9], antifungal [10], antiprotozoal [11], anti-carcinogenic [12], anti-inflammatory [14], antioxidant [14], cardio-protective [15]. It has also been used to inhibit platelet aggregation [16], treat diabetes [17], dyslipidemia, gastrointestinal disturbances [18], anxiety [19], malaria [20], flu, fever, and pneumonia [21], as well as in aromatherapy. In addition to its therapeutic uses, *C citratus* is also consumed as a tea, added to non-alcoholic beverages and baked food, and used as a flavoring and preservative in confections and cuisines. In cosmetics, its essential oils are used as a fragrance in the manufacture of perfumes, soaps, detergents, and creams [22, 23]. In the present study evaluation of its major phytocompounds and antioxidant features have been undertaken.

MATERIALS AND METHODS

Collection of plant materials

Required plant parts were collected from the garden of Govt. V.Y.T. P.G. Auto. College, Durg, Chhattisgarh, India. Healthy plant leaves, stems, and roots were chosen, and samples were placed in sterile sealed plastic bags then brought to the laboratory within a few minutes and then processed. The plant materials-root, bark, stem, and leaf were allowed to dry naturally followed by the grinding process and the powder was kept in well-labeled plastic bottles. 5gm of the material was weighed using an electronic weighing balance. The material was dissolved in 50 ml of distilling water, chloroform, methanol, benzene. The extract was filtered through Whatman filter paper (No. 1) and was stored in sterile bottles for further analysis.

Phytochemical Analysis

Phytochemical analysis of *Neolamarckia cadamba* and *Cymbopogon citratus* was performed from three different parts (Leaf, stem, and Root) extracted in Distilled water, Methanol, Chloroform, and Benzene. All these plant materials were tested for the presence of seven major phytochemical groups like alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, terpenoids.

1. Test for steroids

0.5ml of the extract was dissolved in 3ml of chloroform. The solution was filtered. 2ml of conc. Sulfuric acid was added to the filtrate to form a lower layer. The reddish-brown color ring at the interface indicates the presence of steroids.

2. Test for saponin

0.5ml of the extract was taken in a test tube then 5ml of distilled water was added to it. The solution was vigorously shaken and stable persistence was observed for the presence of saponin.

3. Test for tannins

0.5ml of extract and 5ml of distilled water was taken in a test tube 1% ferric chloride was added. Deep green, brownish-green, or blue coloration indicates the presence of tannin.

4. Test for flavonoids

0.5 ml plant extract and 5ml distilled water was added to the test tube then it was filtered. 5ml of dilute ammonium solution was added to the filtrate then conc. Sulfuric acid was added. The yellow color indicated the presence of flavonoids. The yellow color disappeared on standing.

5. Test for alkaloid

0.5ml extract was taken and 3ml of methanol was added to it. Then 300 microliter of acetic acid was added to it then a solution of ammonium hydroxide was added dropwise. The appearance of precipitate indicated the presence of alkaloids.

6. Test for cardiac glycosides

0.5ml of each extract was treated with 0.2ml glacial acetic acid then dropwise 3.5% ferric chloride was added to the solution. This was layered with 1ml of conc. Sulfuric acid. A reddish-brown ring that has occurred at the interface indicates the presence of cardiac glycosides.

7. Test for terpenoids

0.5ml extract was added to the test tube then 2ml of chloroform was mixed into the solution. 3ml of conc. Sulfuric acid was added to form a lower layer. The occurrence of reddish-brown color at the interface indicates the presence of terpenoids.

Determination of Scavenging Activity

Determination of DPPH radicals scavenging activity was estimated with the method used by 1mM solution of DPPH in methanol and extract solution in ethanol was prepared and different concentration of this solution was added to 1.5 ml of DPPH. The absorbance was measured at 517 nm against the corresponding blank solution which is prepared by taking 3 ml ethanol and control O.D. was prepared by taking 3 ml of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following formula—

$$\text{DPPH scavenged (\%)} = \frac{(\text{A con} - \text{A Test})}{\text{A Con}} \times 100$$

A con- is the absorption of the control
A test- is the absorbance in the presence of the sample of the extracts.

RESULT AND DISCUSSION

Kitaz Adawia *et al*. evaluate the phytochemical constitution and antioxidant activity of methanolic extracts of dried bulbs and aerial parts of selected wild plants in the Liliaceae family growing in Syria *Allium ampeloprasum., Allium stamineum, Asparagus acutifolius, and Ornithogalum umbellatum*. The antioxidant properties of methanol extracts of bulbs and aerial of selected plants were evaluated, through the determination of total phenolics and flavonoids content, as well as DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging. The phytochemical screening revealed the presence of phenols, flavonoids, tannins, saponins, steroids, and terpenoids in bulbs and aerial parts of the studied plant, but none contain coumarins, while cardiac glycosides only present in bulbs of *Allium stamineum*, *Allium ampeloprasum*, and *Ornithogalum umbellatum*. Aerial parts extracts of *Asparagus acutifolius* showed greater DPPH radical scavenging activity (IC_{50} = 0.15), as well as total phenolic (36.10 mg gallic acid equivalent/g of dry weight) and flavonoid content (114.28 mg rutin equivalent/g of dry weight).

M.H. Labiad *et al*., [24] screened phytochemical screening and antioxidant activities in different solvent extracts of *Thymus satureioïdes*. They evaluated extracts for various chemical test for phytochemical constituents, total phenolic contents using the Folin Ciocalteu method and assayed antioxidant activity through *in vitro* radical scavenging activity using DPPH assay, FRAP, and ABTS. They found various phytochemicals like steroids, flavonoids, alkaloids, saponins, and catechuic tannins. The average total phenol content of hydroethanolic extracts was significantly ($P<0.05$) higher when compared with the total polyphenol contents in the hexane, ethyl acetate, and dichloromethane extracts. In order of effectiveness (IC50) of the plant extracts the potent inhibitors were hydroethanolic extract, followed by dichloromethane, ethyl acetate, and the least was the hexane extract, for all the methods (DPPH, ABTS, and FRAP). They found that *Thymus satureioïdes* solvent extracts especially the hydroethanolic extracts may be a potent source of natural antioxidant and its use in the management of diseases associated with oxidative stress is justified.

Ikram Mohamed Eltayeb and Fatima Hashim Mohamed Nari 2017, analyzed phytochemicals and antioxidants from three important medicinal plants; *Aristolochia bracteolata*, *Citrullus colocynthis,* and *Salvia officinalis*. They analyzed ethanolic extracts of the plants, for the presence of different secondary metabolites, and comprised their antioxidant activity. They evaluated antioxidant activity using DDPH radical scavenging assay. They found that all three plants contained cardiac glycosides, terpenoids, and sterols and none contained anthraquinones, carbohydrates, and reducing sugars. The antioxidant activity of *A. bracteolata, C.colocynthis, S. officinalis* extracts were found to be 48.8±0.04, 11.1±0.16, 82.9±0.02 respectively.

In the present study qualitative phytochemical analysis was (Table no. 01 & 02) performed and found the presence of steroids, flavonoids, alkaloids, tannins, saponins, steroids, and cardiac glycosides suggested antioxidant properties that can be used in new drugs for the therapy of oxidative damages. The free radical percentage activity was reported maximum in aqueous extract of stem of *Neolamarckia cadamba* 81% with 1.5 ml (Table no. 06) and minimum percentage of scavenging activity was reported in its aqueous extract of leaf part 3.33 % with 0.5 ml (Table no. 05) whereas methanol extract of stem and leaf showed intermediate activity free radical scavenging activity of 60% and 30% with 1.5 ml (Table no. 04 & 03, Fig.No.1) respectively.

In *Cymbopogon citratus* maximum free radical scavenging percentage activity was reported as 50% with 1.5 ml of methanol extract of leaf part (Table no. 08) followed by 45% with 1.5 ml methanol extract of root part (Table no. 07) and minimum percentage scavenging activity was reported in aqueous extract of stem and leaf part of *Cymbopogon citrates* 1.67% and 1.67% (Table no. 9,10 & 11, Fig.No. 2) respectively.

Table-01: **Showing phytocompounds of *Neolamarckia cadamba* (Leaf, Stem, Bark)**

Phyto-constituents	***Neolamarckia cadamba***											
	Dist. water			**Methanol**			**Chloroform**			**Benzene**		
	Leaf	Stem	Bark	Leaf	Stem	Bark	Leaf	Stem	Bark	Leaf	Stem	Bark
Cardiac Glycosides	–	+	+	-	+	+	-	-	+	-	-	-
Terpenoids	+	+	-	+	+	-	+	+	-	-	+	-
Steriods	+	+++	+	+	+++	+	+	-	-	+	-	-
Saponin	-	-	-	-	-	-	-	-	-	-	-	-
Tanins	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloid	+	+	-	+	-	-	-	-	-	-	-	-

(+++) indicates higher conc, (++) indicate moderate conc, (+) low conc, (-) indicates negative results

Table-02: Showing phytocompounds of *Cymbopogon citratus* (Leaf, Stem, Root)

Phyto-constituents	*Cymbopogon citratus*											
	Dist. water			Methanol			Chloroform			Benzene		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Cardiac Glycosides	-	-	+	-	+	+	-	-	+	-	-	+
Terpenoids	++	+	-	-	+	+	-	+	-	-	+	-
Steriods	+++	+	-	+	+	-	-	-	-	-	-	+
Saponin	-	-	+	-	++	-	-	-	-	+	-	-
Tanins	-	-	-	-	-	+	-	-	-	+	-	-
Flavanoids	-	-	-	+	-	-	-	-	-	+	-	-
Alkaloid	+	++	++	-	-	-	-	-	-	-	-	+

(+++) indicates higher conc, (++) indicate moderate conc, (+) low conc, (-) indicates negative results

Table-03: Showing DPPH assay of *Neolamarckia cadamba* methanol leaf extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.56**	6.67
1	**1.5**	**0.5**	**0.50**	16.67
1.5	1.5	**0**	**0.42**	30.00

Table-04: Showing DPPH assay of *Neolamarckia cadamba* methanol stem extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.32**	46.67
1	**1.5**	**0.5**	**0.28**	53.33
1.5	1.5	**0**	**0.24**	60.00

Table-05: Showing DPPH assay of *Neolamarckia cadamba* aqueous leaf extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.58**	3.33
1	**1.5**	**0.5**	**0.52**	13.33
1.5	1.5	**0**	**0.48**	20.00

Table-06: Showing DPPH assay of *Neolamarckia cadamba* aqueous stem extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.30**	50.00
1	**1.5**	**0.5**	**0.16**	73.33
1.5	1.5	**0**	**0.11**	81.67

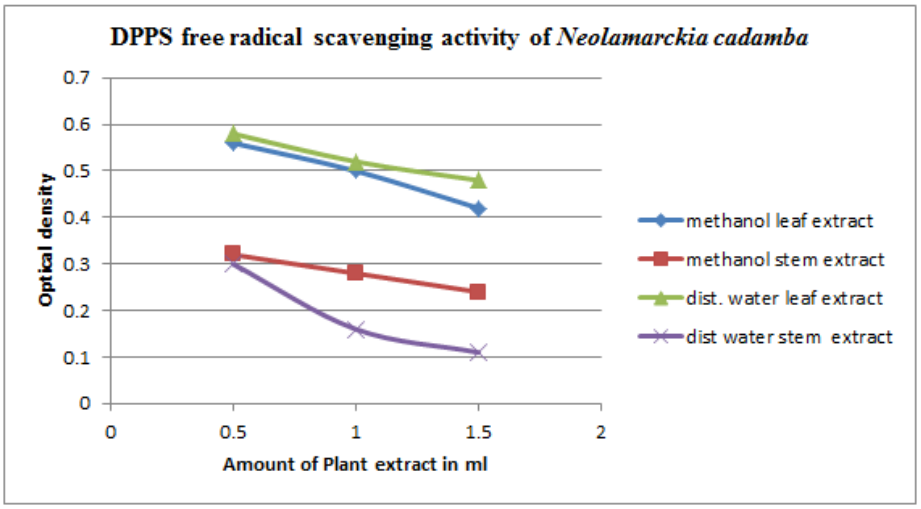

Fig-1: Showing Antioxident features of *Neolamarckia cadamba*

Table-07: Showing DPPH assay of *Cymbopogon citratus* methanol root extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.38**	36.67
1	**1.5**	**0.5**	**0.35**	41.67
1.5	1.5	**0**	**0.33**	45.00

Table-08: Showing DPPH assay of *Cymbopogon citratus* methanol leaf extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.34**	43.33
1	**1.5**	**0.5**	**0.32**	46.67
1.5	1.5	**0**	**0.30**	50.00

Table-09: Showing DPPH assay of *Cymbopogon citratus* aqueous root extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.56**	6.67
1	**1.5**	**0.5**	**0.54**	10.00
1.5	1.5	**0**	**0.52**	13.33

Table-10: Showing DPPH Assay of *Cymbopogon citratus* aqueous stem extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.59**	1.67
1	**1.5**	**0.5**	**0.58**	3.33
1.5	1.5	**0**	**0.42**	30.00

Table-11: Showing DPPH Assay of *Cymbopogon citratus* aqueous leaf extract

PLANT EXTRACT	DPPH	METHANOL	OPTICAL DENSITY	SCAVENGING% (In %)
0.5	**1.5**	**1**	**0.59**	1.67
1	**1.5**	**0.5**	**0.57**	5.00
1.5	1.5	**0**	**0.52**	13.33

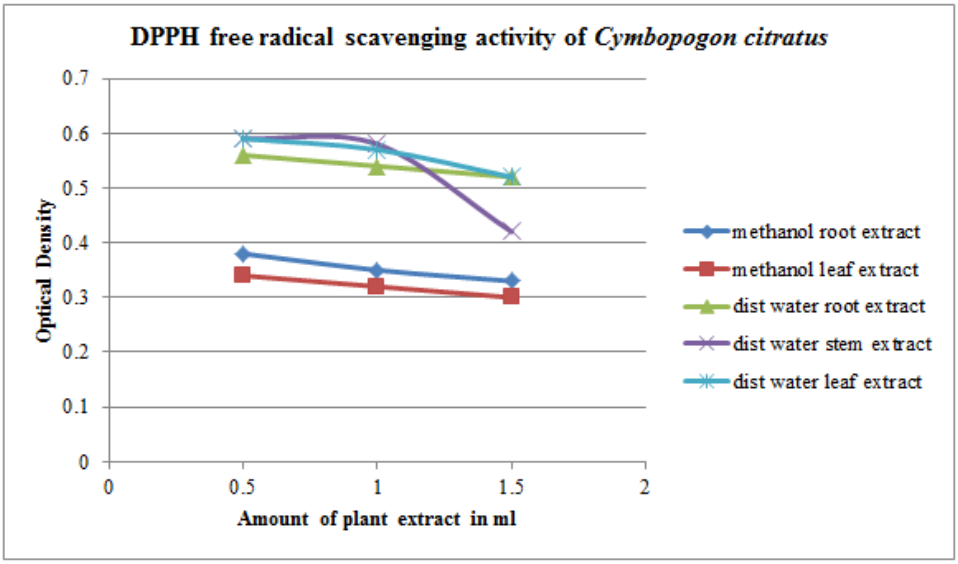

Fig-2: Showing Antioxiden features of *Cymbopogon citratus*

CONCLUSION

Our findings are affirmative to the line of previous work and confirm that both plants having several metabolites of pharmaceutical importance. Its good antioxidant features may signify its application for pharmaceuticals as well as nutraceuticals.

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