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ORIGINAL ARTICLE

Comparative Phytochemical Evaluation of the Aerial Parts of *Neolamarckia cadamba* (Roxb.) Bosser

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Neolamarckia cadamba (Roxb.) Bosser is an important traditionally using medicinal plant. Almost all parts of plants are used for medicinal purposes against numerous diseases. In this present study, presence of secondary metabolites was compared between bark, leaves, ripe and unripe fruits of plant using preliminary phytochemical screening using standard methods in solvents having different degree of polarity- acetone, benzene, methanol, petroleum ether and water. Comparative study indicated that polar solvents were superior in extracting secondary metabolites as compared to non- polar. This present study also suggested leaves were high on secondary metabolites followed by ripe fruits and bark whereas unripe fruits found to be on lower level on major classes of secondary metabolites.

Key words: Phytochemicals, Neolamarckia cadamba, Secondary metabolites, polar solvents, Antioxidants

Plants are rich source of phytochemical which plays key role in maintaining human health Phytochemicals comprises of bioactive compounds which are known to have therapeutic activities against various ailments. They are the most uncomplicated and best source for obtaining natural drugs with numerous biological activities. These bioactive compounds can differ in plants or in their plant parts and can be used as drugs for the treatment of illness either alone or in combination with other plants. Preliminary phytochemical screening plays an important role in evaluation of these bioactive compounds.

Polyphenols are one of the most widely distributed secondary metabolites in plant kingdom ranging from simple phenolic acid to tannins, highly complex compound and are also found to be the most in human diets (Li *et al*., 2006). Indole alkaloids are also found to be widely distributed secondary metabolites groups in plants of Apocynaceae, Rubiaceae, Nyssaceae and Loganiaceae families. Respine, antihypertensive drug, Vinblastin & Vincristine, antitumor drug are one of the most important Indole alkaloids has been isolated from the plants (Sagi *et al*., 2016; El-Sayed and Verpoorte, 2007). Phenolic glycoside is also one of the abundant secondary metabolites found to have major role in plant defence mechanism against herbivores (Boeckler *et al*., 2011). Precisely phytochemical evaluation provides the base for isolation or extraction of targeted compounds which helps in investigation of various biological activities (Shaikh and Patil, 2020), as previously reported two novel indole alkaloids, Neolamarckines A and B only found in *N. cadamba* (Qureshi *et al*., 2011).

Neolamarckia cadamba (Roxb.) Bosser is a traditional medicinal plant belonging to family Rubiaceae. Several Plant parts of *N. cadamba* have been found possessing various secondary metabolites and are used in treatment of various ailments (Umachigi *et al*., 2007). *N. cadamba* reported to be used against fever, vomiting, cough, snake bite, wounds, diuresis, burning sensation, ulcers, menorrhogia, skin diseases, semen quality, diarrhoea, stomatitis and uterine complaints (Dubey *et al*., 2011). *N. cadamba* also possess numerous pharmacological activities like antimalarial, antifungal, antidiabetic, antioxidant, antilithic, antibacterial, antihepatotoxic, antilipidemic, anti- inflammatory and analgesic activity(Kapil *et al*., 1995; Bussa and Jyothi, 2010; Jeyalalitha *et al*., 2013; Mishra and Siddique, 2011; Pant *et al*., 2012). Medicinal properties of *N. cadamba* have also been mentioned in ancient Indian Ayurveda medicinal literatures.

MATERIALS AND METHODS

Collection

Healthy and fresh bark, leaves and both ripe and unripe fruits were collected from Ranchi district of Jharkhand. Bark and leaves were washed and shade dried for a week whereas fruits were washed and oven dried at 60^0 C. All dried samples were made into powder form using grinder and mortar- pestle.

Extract preparation

Dried samples were then extracted with different solvents (1: 10 w/v) and then filtered through whatman filter paper. Filterates were then kept at room temperature till concentrated crude extract was obtained.

Phytochemical analysis

Phytochemical screening was performed in both polar and non-polar solvent (acetone, benzene, methanol, petroleum ether and distilled water) for the presence of various phytochemicals in plant extract as per the different standard methods.

Test for carbohydrates

Molisch's test: 2 ml extract was treated with few drops of molisch's reagent and then with 2ml of conc. Sulphuric acid. Formation of red or reddish violet ring confirms the presence of carbohydrates.

Benedict's test: Extract was treated with Benedict's reagent and then heated on a water bath for approx. 5 min, cooled and then brown precipitate was observed which confirms the presence of carbohydrates.

Test for sterols

Salkowski test: 2ml of extract was treated with 2 ml of conc. H_2SO_4. Red or brownish red layer was observed which shows the presence of sterols.

Test for alkaloids

The small quantity of extract was treated with hydrochloric acid until ppt. formation and then filtered. Filterates were used for different alkaloid detection test.

Dragendorff's test: Filtered extract was treated with drops of dragendorff's reagent. Appearance of reddish brown ppt. confirms the presence of alkaloids

Mayer's test: Extract was treated with few drops of mayer's reagent. Appearance of creamy ppt. shows the presence of alkaloids.

Wagner's test: Extract was treated with wagner's reagent. Appearance of brownish ppt. shows the presence of alkaloids.

Test for Flavonoids:

Small quantity of the extract with Ethyl acetate was heated in water bath for approx. 4 min. and then filtered. Filtrates were used for different flavonoids detection tests.

Ammonium test: Filtered extract was treated with 1 ml of dil. Ammonium hydroxide (1%, v/v). Yellow coloured layer was observed which shows the presence of flavonoid.

Shinoda test: Few magnesium ribbons and few drops of conc. Hydrochloric acid were added to filtered extract. Appearance of red colour confirms the presence of flavonoids.

Alkaline reagent test: Filtered extract was treated with few drops of sodium hydroxide solution (20%, w/v). Appearance of fluorescent yellow confirms the presence of flavonoids which turns colourless with addition of dil. Hydrochloric acid.

Test for tannins

Lead acetate test: In 2 ml of plant extract lead acetate solution (10%, w/v) was added white ppt. was observed which shows the presence of tannins.

Ferric chloride test: In 2 ml of plant extract few drops of ferric chloride solution was added (5%, w/v). Appearance of bluish black colour confirms the presence of tannins.

Potassium dichromate test: Appearance of dark colour occurs when potassium dichromate solution was added. It shows the presence of tannins.

Test for cardiac glycosides:

Keller Killiani test: 0.4 ml of glacial acetic acid and a drop of 5% ferric chloride solution were added to extract. The 0.5 ml of conc. Sulphuric acid was added. Formation of reddish brown or bluish brown indicates the presence of tannins.

Legal test: 2ml of extract was treated with 1ml of pyridine and 1ml sodium nitroprusside solution. Alkaline red or pink colour was observed which confirms the presence of cardiac glycosides.

Test for Anthraquinone Glycosides:

Borntrager's test: 5 ml of extract was treated with dil. Hydrochloric acid and 5 ml of 5% ferric chloride and then heated on water bath for few min. and then cooled down. Benzene was added to mixture and shaken well. Benzene layer separates from the mixture and then dil. Ammonium hydroxide was added which produces pink colour which confirms the presence of anthraquinone glycosides.

Test for Phenol:

$FeCl_3$ test: When 2 ml of extract was treated with few drops of ferric chloride (10%, w/v). Dark green colour appeared which indicates the presence of phenol.

Test for proteins:

Biuret test: 4% sodium hydroxide is added to extract solution followed by 1% $CuSO_4$. Appearance of violet colour shows the presence of proteins.

Xanthoproteic test: Conc. sulphuric acid is added to extract solution. Upon formation of ppt. it is heated on boiling water bath. Ammonium hydroxide is then added. Appearance of orange or reddish orange confirms the presence of protein.

Test for amino acid

Ninhydrin test: Few drops of 5% Ninhydrin solution was added in extract solution and heated on boiling water bath for approx. 10 min. Appearance of blue or purple colour shows the presence of amino acid.

Test for Quinones

HCL test: 2ml of extract was treated with dil. Hydrochloric acid and then heated in boiling water bath and then cooled down. Chloroform was added to cooled solution which forms the separate layer which is then

collected. To which ammonia is added which gives purple or red colour which signifies the presence of quinone.

Test for coumarin

NaOH test: 2ml of extract was treated with 3ml of 10% sodium hydroxide. Appearance of yellow colour shows the presence of coumarin..

Test for anthocyanins

Conc. H_2SO_4 test: Extract solution was treated with conc. sulphuric acid. Appearance of orange to yellow colour shows the presence of anthocyanin.

Test for fats & oils

Solubility test: Extract was dissolved in ethanol, chloroform and ether and then miscibility of extract was observed. If it is soluble in above it indicates the presence of fat and oils.

RESULTS AND DISCUSSION

Solvents of different polarities used in this study were acetone, benzene, methanol, petroleum ether and water. A preliminary phytochemical was performed using different standard methods (Sofowora, 1996; Khandelwal, 2008; Kaushik and Sharma, 2012). This phytochemical screening comparatively demonstrates the presence and absence of carbohydrates, sterols, alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinone glycosides, phenols, proteins, amino acid, quinones, coumarins anthocyanin and fats & oils in the present study of phytoconstituents (bark, leaves, ripe and unripe fruits) in Table1.. Alkaloid was observed in abundant comparatively to other secondary metabolites in wagner's reagent test in all four plant parts. It was also observed that alkaloid was specifically abundant in ripe fruits followed by barks and unripe fruits. Poor intensity of flavonoid was observed in both fruits, absent in bark but present in leaves. Previous works on different plant have also revealed poor performance of fruits in levels of flavonoids and polyphenols as compare to leaves (Wang *et al*., 2012; Brahmi *et al*., 2013; Mocan *et al*., 2014). Phenol was also found to be in good amount in leaves along with coumarins comparatively to the rest of the studied phytoconstituents than the anthocyanin which was found negligible in unripe fruits and leaves but abundant in acetone and methanolic extracts of bark and ripe fruits. According to study accumulation of anthocyanin occurs in young and juvenile leaves whereas degradation occurs as leaves starts to mature which can be due to degrading enzymes like peroxidases, β- glycosidases and development & environment condition as well (Oren-Shamir, 2009). Previous reports on phenol have confirmed that fruits that are rich in phenol make them high in antioxidant which helps in balancing of lipoprotein benefitting against the cardiac diseases (Vinson *et al*., 2001). Tannins were also found high in leaf extracts than bark and fruits. Cardiac glycoside was observed in minimal amount in all four phytoconstitutes and was also observed in works of other researchers on *N. cadamba* as well (Golla *et al*., 2018; Kumar *et al*., 2020). Although Cardiac glycoside is known to be poisonous secondary metabolite, they are also used in treatment of cardiac complaints (Dobler *et al*., 2015). According to study levels of anthraquinone glycoside determines the intensity of laxative properties in plants (Sakulpanich and Gritsanapan, 2009) which were found to be in very small amount in leaves and absent in fruits. Fats & Oils were present in moderate amount in all four phytoconstitutes whereas quinone was found to be completely absent in bark and poorly in both ripe and unripe fruits. Pandey *et al*. (2018) studied the nutraceutical properties of *N. cadamba* fruits suggested that nectars of *N. cadamba* can be used as primary food and can be fit for consuming due to their richness in antioxidants and minerals.

Among all the five solvent extracts, most of the secondary metabolites appeared in acetone and methanolic extracts followed by others. Petroleum ether displayed the poorest performance in extracting secondary metabolites. This indicates that polar solvents are more effective in extracting secondary metabolites. This may be due to poor infiltration of non- polar solvents in tissues of plants for the extraction of secondary metabolites vice versa, although traditional healers generally prefer water to prepare extraction for the medicinal purposes (Sarath and Sudha, 2019).

Table 1: Preliminary phytochemical screening of bark, leaves, ripe and unripe fruits.

S. No.	Chemical test/ Solvent	BA	L	RF	UF
1.	**Carbohydrates**				
I.	Molisch's test				
a)	A	+++	++	+++	+++
b)	B	+	+	-	++
c)	M	+++	-	+++	++
d)	PE	-	+++	-	-
e)	W	-	+	++	-
II.	Benedict's test				
a)	A	-	++	+	+
b)	B	+	+	-	-
c)	M	-	-	-	-
d)	PE	-	+++	-	-
e)	W	-	-	-	-
2.	**Sterols**				
I.	Salkowski's test				
a)	A	+++	+++	+++	+++
b)	B	-	-	-	-
c)	M	+++	+++	-	+
d)	PE	-	-	-	-
e)	W	+	++	-	-
3.	**Proteins**				
I.	Biuret test				
a)	A	-	-	-	-
b)	B	+++	+++	+++	-
c)	M	-	++	-	-
d)	PE	-	-	-	-
e)	W	+	++	++	++
II.	Xanthoproteic test				
a)	A	+	++	+	+
b)	B	-	+	++	-
c)	M	+	+++	-	-
d)	PE	+	-	-	-
e)	W	-	+	+	-
4.	**Alkaloids**				
I.	Dragendorff's test				
a)	A	-	-	-	-
b)	B	-	-	-	-
c)	M	-	+++	-	-
d)	PE	-	+	-	-
e)	W	-	-	-	-
II.	Mayer's test				
a)	A	+	-	-	-
b)	B	+	-	+	+
c)	M	-	-	+	+
d)	PE	-	-	+	+
e)	W	+	-	+	+
III.	Wagner's test				
a)	A	++	++	+++	+++
b)	B	+++	+	+++	+++
c)	M	+++	+	+++	++
d)	PE	++	+	+++	+++
e)	W	+++	+	+++	+++
5.	**Flavonoids**				
I.	Ammonium test				

a)	A	-	+	++	++
b)	B	-	-	-	-
c)	M	-	+	-	-
d)	PE	-	+	-	-
e)	W	-	+	-	-
II.	Alkaline reagent test				
a)	A	-	+++	++	++
b)	B	-	++	-	-
c)	M	-	-	-	+
d)	PE	-	+	-	-
e)	W	-	-	-	-
III.	Shinoda test				
a)	A	-	-	+	+
b)	B	-	+	-	-
c)	M	-	++	-	-
d)	PE	-	-	-	-
e)	W	-	-	-	-
6.	**Tannins**				
I.	Lead acetate test				
a)	A	+	+++	-	-
b)	B	+++	++	+	-
c)	M	+	+++	++	++
d)	PE	-	+++	-	-
e)	W	+++	+	++	+
II.	Ferric chloride test				
a)	A	+++	+++	++	++
b)	B	++	++	++	-
c)	M	++	+++	++	+++
d)	PE	-	+	-	-
e)	W	+	++	+	+++
III.	Potassium dichromate test				
a)	A	+	-	+	+
b)	B	++	+++	++	-
c)	M	-	-	-	-
d)	PE	-	+	-	-
e)	W	+++	++	++	++
7.	**Cardiac glycosides**				
I.	Keller- Killiani test				
a)	A	+	+	+	+
b)	B	+	+	-	+
c)	M	-	+	+	+
d)	PE	-	+	-	-
e)	W	+	+	+	+
II.	Legal test				
a)	A	-	-	-	+
b)	B	++	+	-	-
c)	M	+	-	+	+
d)	PE	-	-	-	-
e)	W	++	+	+	++
8.	**Anthraquinone glycoside**				
I.	Borntager's test				
a)	A	-	-	-	-
b)	B	+	-	-	-
c)	M	-	-	-	-
d)	PE	-	+	-	-
e)	W	-	++	-	-
9.	**Phenols**				

I.	$FeCl_3$ test				
a)	A	+++	++	++	++
b)	B	++	+	++	-
c)	M	++	+++	++	+++
d)	PE	-	+	-	-
e)	W	+	+	+	+++
10.	**Amino acids**				
I.	Ninhydrin test				
a)	A	-	+	-	-
b)	B	-	+	+	-
c)	M	+	+	-	-
d)	PE	-	-	-	-
e)	W	-	+	-	+
11.	**Quinones**				
I.	HCl test				
a)	A	-	++	++	++
b)	B	-	+	-	-
c)	M	-	+	-	-
d)	PE	-	++	-	-
e)	W	-	+	-	-
12.	**Coumarin**				
I.	NaOH test				
a)	A	-	++	++	++
b)	B	-	+	-	-
c)	M	-	+	-	-
d)	PE	-	++	-	-
e)	W	-	+	-	-
13.	**Anthocyanin**				
I.	Conc. H_2SO_4 test				
a)	A	+++	-	+++	+
b)	B	-	-	-	-
c)	M	+++	-	+++	-
d)	PE	+	-	-	-
e)	W	-	-	++	-
14.	**Fats & Oils**				
I.	Solubility test				
a)	A	+	+	++	++
b)	B	-	-	-	-
c)	M	+	++	++	++
d)	PE	++	++	++	++
e)	W	-	-	+	+

BA- Bark, **L-** Leaves, **RF-** Ripe fruits, **UF-** Unripe fruits

A- Acetone, **B-** Benzene, **M-** Methanol, **PE-** Petroleum ether, **W-** Water

CONCLUSIONS

Different extracts of studied plant parts revealed the phytochemicals in the form of secondary metabolites. This phytochemical analysis provides the evidence of using plant as drugs and confirms its traditional usages against various diseases. Being an herbal plant, drugs will have minimal to nil side effects and also it is important to determine the therapeutic use of plant drug via scientific medium.

Overall it was concluded that out of all the four studied phytoconstituents, leaves were abundant in secondary metabolites followed by ripe fruits, bark and then unripe fruits which also supports the previous report by Ganjewala *et al*. (2013).

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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