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ANTIOXIDANT AND ANTICANCER POTENTIAL OF *Neolamarckia cadamba* (ROXB.) BARK EXTRACT

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KEYWORDS

N. cadamba

DPPH

Sulforhodamine

N1S1 rat hepatoma cell line

ABSTRACT

Current study was conducted to screen the antioxidant and anti cancerous potential of Hydro-methanolic (HM) bark extract of *Neolamarckia cadamba*. Further presence of various phytochemicals in HM extract of *N. cadamba* was also accessed by using suitable phytochemical tests. Result of current study suggested the presence of alkaloids, steroids, glycosides, triterpenoids, carbohydrates, flavonoids, tannins and phenols where as the presence of protein and amino acids was not reported in the HM extract of *N. cadamba*. *In vitro* antioxidant potential of different doses i.e. 0.025, 0.05, 0.1 and 0.2 mg/ml of *N. cadamba* HM bark extract were determined using DPPH free radical scavenging method. Results showed significant ($p < 0.01$) antioxidant efficacy of 0.025, 0.05 and 0.1 mg/ml doses of extract when compared with standard treatment. Evaluation of anticancer activity of different concentrations (10, 20, 40 and 80 µg/ml) of HM extract was done against N1S1 rat hepatoma cancerous cell line using sulforhodamine (SRB) assay. Percentage of control cell growth was -37.66 and -34.13 at 40 µg/ml and 80 µg/ml respectively. Dose dependent decrease in percentage of control cell growth was observed. LC50, TGI and GI50 of HM extract was found to be 75.92, 46.73 and 17.46 µg/ml respectively. Present study concludes *in vitro* antioxidant and anticancerous potential of HM extract of *N. cadamba* bark.

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1 Introduction

Neolamarckia cadamba synonyms Kadam, native to South Asia and South East Asia (Krisnawati et al., 2011). The Charaka Samhita and Sushruta Samhita narrates the ethnomedicinal uses of *N. cadamba* bark as an analgesic, for strengthening body, against snake bite, diarrhea, dysentery, leucorrhoea and spermatorrhoea (Sircar, 1992; Sikar et al., 1992). Further, various parts of *N. cadamba* i.e. leaf, bark, fruit and flower has been traditionally used by many tribes and communities in India against fever, cough, sour throat, infections and inflammation (Pandey & Negi, 2016). Phytochemical analysis of different parts of *N. cadamba* detects the presence of chlorogenic acid, β -sitosterol, indole alkaloids and cadambine which shows anti-inflammatory, antimutagenic, antipyretic, antidiabetic, antidiarrheal and antioxidant activities (Gupta et al., 1980; Li & Chang, 2005; Alam et al., 2008; Kumar et al., 2015a; Verma et al., 2019; Mondal et al., 2020).

Methanolic extract of *N. cadamba* bark contains number of secondary metabolites such as cadambagenic acid, saponin B, Glycosides A & B, Phelasin A & B and 3 β -dihydrocadambine (Sahu et al., 1999; Sahu et al., 2000; Chandel et al., 2011). Presence of phenolics compounds, alkaloids and saponins in different extracts of *N. cadamba* bark attributes to its antimicrobial, antidiabetic, antiepileptic activity (Gurjar et al., 2010; Patel et al., 2011; Nagakannan et al., 2011). Chronic persistence of free radicals (reactive oxygen species; ROS and reactive nitrogen species; RNS) lead to oxidative stress, responsible for disorders related to nervous system, metabolic disorders like diabetes mellitus and several form of cancers (Huy et al., 2008). Traditionally bark of *N. cadamba* has been used against inflammation, fever, cough and wound. To scientifically validate ethno-medicinal uses of bark of *N. cadamba*, present study has been designed to determine *in vitro* antioxidant and anticancer efficacy of hydromethanolic (HM) extract of *N. cadamba* bark.

2 Materials and Methods

2.1 Used chemicals and cell line used Experimental animals

2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, butylated hydroxyl toluene (BHT), RPMI-1640 medium, sulforhodamine (SRB) and TCA were procured from Sigma Aldrich, USA. N1S1 rat hepatoma cancerous cell line was obtained from Tata Memorial centre, Mumbai.

2.2 Plant material

Neolamarckia cadamba bark was collected from Mathura and Vrindavan areas of Uttar Pradesh, India. Plant was identified and authenticate by Dr A S Upadhye, Agharkar Research Institute, Pune with voucher deposition No. L-084. Dried bark powder of *N.*

cadamba was used in preparation of hydro-methanolic (HM) extract.

2.3 Extract Preparation

Dried powder of *N. cadamba* bark was subjected to soxhlet extraction. Hydro-methanolic (80:20, v/v) solvent was allowed to boil at 65° C for 7-8 hrs. Solvent was evaporated at temperature and reduced pressure using rotary evaporator. Lastly dark brown crystals were obtained and used in further studies.

2.4 Preliminary Phytochemical analysis of HM extract of *N. cadamba* bark

Qualitative analysis of hydro-methanolic extract of *N. cadamba* bark was done as per standard protocol (Debela, 2002). HM extract of *N. cadamba* bark were subjected to preliminary phytochemical screening to detect the presence of steroids, triterpenoids, glycosides, carbohydrates, alkaloids, flavonoids, tannins, phenolics, proteins, amino acids, gum, mucilages, fats and oils.

2.5 Evaluation of *in vitro* antioxidant potential of HM extract of *N. cadamba* bark

In vitro antioxidant activity of HM extract of *N. cadamba* bark was evaluated by 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay method (Blois, 1958). Different concentrations (0.025 mg/ml, 0.05 mg/ml, 0.1 mg/ml and 0.2 mg/ml) of HM extract of *N. cadamba* bark were used for antioxidant activity. From various concentrations, 0.1 ml HM extract were mixed with 3.0 ml of DPPH (0.1 mM). Mixture was incubated at 20° C for 40 minutes in dark. Optical density (OD) was determined at 517 nm with ethanol and water (1:1) as blank. Readings were taken in triplicate. Determination of percentage inhibition of DPPH was calculated by using formula given by Gulcin et al., (2005).

$$\text{Percent inhibition of DPPH} = \frac{\text{OD}_{\text{control (DPPH)}} - \text{OD}_{\text{test (HM extract)}}}{\text{OD}_{\text{control (DPPH)}}} \times 100$$

Standard used were butylated hydroxyl toluene (BHT) and ascorbic acid were used as standards.

2.6 *In vitro* anticancer activity of HM extract of *N. cadamba* bark

Determination of *in vitro* anticancer activity of HM extract of *N. cadamba* bark was carried out at Tata Memorial centre, Mumbai using N1S1 rat hepatoma cell line according to standard procedure (Vichai & Kirtikara, 2006). Cell suspension (100 μ L) in triplet was inoculated in 96 well microtiter plate. Plate was incubated at 37° C in CO₂ incubator (5% CO₂ & 100% RH) for 24 hrs. Different doses (10, 20, 40 & 80 μ g/ml) of HM extract and Adriamycin (standard) were added into wells of microtiter plate. Plate was

again allowed to incubate at 37 °C for 48 hours in CO₂ incubator. Termination of reaction was done by adding 30% chilled TCA. This was followed by staining of cells by adding 0.4% sulforhodamine (SRB) in 1% acetic acid solution. Elution of SRB dye bounded to cell protein was carried out by adding Tris base. Absorbance was measured at 510 nm by ELISA reader.

Percent growth = Mean absorbance test / Mean absorbance control × 100

$$\text{Percent of control cell growth} = \frac{\text{Mean absorbance sample} - \text{mean absorbance day 0}}{\text{Mean absorbance neg control} - \text{mean absorbance day 0}} \times 100$$

Percent growth inhibition = 100 - % of control cell growth

Percent cell killed = 100 - mean absorbance sample / mean absorbance day 0 × 100

2.7 Statistical analysis

Data analysis was done using one way analysis of variance (ANOVA) using SPSS version 20.0 software and DMRT at $p < 0.01$ to determine significant differences among treated means. Values are expressed as mean ± SEM.

3 Results and Discussion

Percentage yield of *N. cadamba* HM extract was reported 14 percent. Results of preliminary phytochemical analysis revealed the presence of alkaloids, steroids, glycosides, triterpenoids, carbohydrates, flavonoids, tannins, phenols, fats, oils, gums, mucilage's where as proteins and amino acids were absent (Table 1). Presence of these secondary metabolites cause inhibition of microbial growth (Negi, 2012) and does offer various ethnomedicinal uses of *N. cadamba* bark (Pandey & Negi, 2016).

Present study concerns with evaluation of *in vitro* antioxidant potential of *N. cadamba* bark extract. DPPH free radical scavenging method was used to determine percentage inhibition of free radicals in presence of 0.025, 0.050, 0.1 and 0.2 mg/ml HM extract of *N. cadamba* bark. Results of study suggested significant ($p < 0.01$) antioxidant efficacy of *N. cadamba* bark extract at different doses, when compared with ascorbic acid and BHT (Table 2).

Maximum percentage inhibition was reported at 0.20 mg/ml HM extract. Dose dependent inhibition of free radical scavenging activity was found. Result of current study are in agreement with previous findings, suggesting the presence of phenolics compounds in bark extract might caused scavenging of free radicals (Chandel et al., 2011). Antioxidant compounds counter free radicals mediate oxidative stress in the cell (Blokhina et al., 2003; Mondal et al.,

Table 1 Phytochemical screening of HM extract of *N. cadamba* bark

SN	Phytochemical	Test Name	Inference
1	Alkaloids	Mayer's, Hager's and Wagner's	+
2	Steroids	Salkowski's and Liberman Burchard's	+
3	Glycosides	Legal's and Borntrager	+
4	Triterpenoids	Salkowski's	+
5	Carbohydrates	Molisch's	+
6	Proteins and amino acids	Ninhydrin	-
7	Flavonoids	Shinoda and Alkaline reagent	+
8	Tannins and Phenols	5% FeCl ₃ and Bromine water	+
9	Fats and oils	Saponification	+
10	Gum and Mucilage's	Ruthenium red	+

+ sign indicates present and - sign indicates absent

Table 2 Percentage inhibition of free radicals in presence of different concentrations of HM extracts, Ascorbic acid and BHT

S.N	Concentration (mg/ml)	HM extract	Ascorbic acid (Standard)	BHT (Standard)
I	0.025	71.21 ^c ± 0.96	12.56 ^a ± 0.16	19.72 ^b ± 0.71
II	0.050	78.86 ^c ± 1.02	24.31 ^a ± 0.97	43.25 ^b ± 0.16
III	0.10	82.73 ^c ± 1.05	42.82 ^a ± 0.30	55.74 ^b ± 1.07
IV	0.20	84.13 ^b ± 0.95	93.37 ^c ± 0.02	75.88 ^a ± 0.80

Values represent mean ± SEM of triplet experiment. Results are significant at $p < 0.01$

2020). Present study also revealed the presence of phenols during phytochemical screening of *N. cadamba* bark, which might probably responsible for its significant antioxidant activity. Result of current study can be concluded that HM extract of *N. cadamba* is good enough to overcome oxidative stress and serves as therapeutic agents for treating radical related pathological damage.

Four concentrations (10, 20, 40 and 80 µg/ml) of HM extract of *N. cadamba* bark were screened using N1S1 rat hepatoma cell line. Adriamycin was used as standard cytotoxic agent. Different concentrations of HM extract of *N. cadamba* bark were found to be effective against N1S1 rat hepatoma cell line (Table-3). Dose dependent decrease in percentage cell growth of rat hepatoma was reported in current study. LC₅₀, TGI and GI₅₀ of HM extract was reported 75.92, 46.73 and 17.46 µg/ml respectively (Table 4). Previous studies also suggested cytotoxic activity of *N. cadamba* against different human cancer cell lines (Singh et al., 2013;

Table-3 Effect of different concentrations of HM extracts against % Cell growth of N1S1 rat hepatoma cell line

% Control Growth of rat hepatoma cell line N1S1					
S.N	Extract/Drug	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml
1.	HM extract	90.50	48.06	-37.66	-34.13
2.	ADR (Adriamycin)	-14.21	-31.84	-46.75	-17.92

Table 4 LC50, TGI and GI50 of HM extract and ADR

Rat hepatoma Cell line N1S1	Drug Concentrations (µg/ml)		
N1S1	LC50	TGI	GI50
HM extract	75.92	46.73	17.46
ADR	NE	<10	<10

LC50 - concentration of drug causing 50% cell kill; TGI - concentration of drug causing total inhibition of cell growth; GI50 - concentration of drug causing 50% inhibition of cell growth; ADR - adriamycin standard, HM extract - hydromethanolic extract

Kumar et al., 2015b; Dolai et al., 2016). Current study depicts the presence of phenolic compounds in HM extract of *N. cadamba* bark, responsible for antiproliferative activity (Fatima et al., 2016).

Conclusion

Present study concluded significant antioxidant activity of different doses of HM extract of *N. cadamba* bark. Study also revealed anticancer efficacy of bark extract. There is a need to isolate and molecular characterization of active ingredients present in hydro-methanolic extract of *N. cadamba* bark.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

Author's contribution

Vishal Khandelwal and Pradeep Kumar Choudhary contributed significantly and equally.

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Data Availability

All datasets generated or analyzed during this study are included in the manuscript.

Ethics Statement

Ethical clearance was obtained with IAEC approval vide GLAIPR/CPCSEA/IAEC/2014/Biotech/02.

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