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Antioxidant, angiotensin converting enzyme inhibitory and anthelmintic activities of bark essential oil of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet from North East India

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Abstract. *Cinnamomum bejolghota* (Buch.-Ham.) Sweetisa traditional anthelmintic and cardiotonic medicinal plant. The essential oil extracted from the bark of *C. bejolghota* was characterized by gas chromatography-mass spectrometry and 28 constituents were identified. Monoterpenoids were found to be dominating chemical constituents with α-terpineol (18.57 %) as the major component of the essential oil. The antioxidant activity of the essential oil was evaluated by DPPH radical, β-carotene bleaching and reducing power assay methods. The essential oil showed *in-vitro* dose-response hindering activity against angiotensin-converting enzyme with an IC_{50} value of 33.43 ± 0.46 μg/mL. The essential oil also exhibited potent anthelmintic property *in-vitro* against *Caenorhabditis elegans* with an ED_{50} value of 80.53 ± 3.57 μg/mLin adult mortality assay. The inhibitory activity of essential oil against larval development assay showed an ED_{50}value of 72.40 ± 2.68 μg/mL against *C. elegans* larva. Further, the egg hatching assay using *C. elegans*eggs demonstrated an ED_{50} value of 68.27 ± 3.51 μg/mL with this essential oil. Thus, the ethnomedicinal claim has been validated for the bark essential oil, which provides an excellent scope for the development of the *C. bejolghota* bark essential oil as novel herbal antihypertensive and anthelmintic agents.

Keywords: Cinnamomum bejolghota, bark essential oil, antioxidant, angiotensin-converting enzyme inhibition, anthelmintic activity.

Classification numbers: 1.4.6, 1.2.1

1. INTRODUCTION

North East India possesses two mega phytogeographical zones and among these ones is the Eastern Himalaya and the other is the North Eastern states. This mega centre of the Indian sub-

continent biodiversity covers 50 % of the total flora in India. Various ethnic communities in this geographical region depend mostly on the local herbs for their primary health care and thereby have a good knowledge on medicinal usage of unexplored flora [1]. Among them, *Cinnamomum bejolghota* (Buch-Ham.) Sweet (syn. *C. obtusifolium* Nees.) (Lauraceae) is an evergreen tree. Itis found in the sub-Eastern Himalayan range at an altitude of 2100 m. The plant is found widely distributed in Assam, Nagaland and Meghalaya states of North East India. The stem, bark and leaves of the plant are aromatic and used as a spice in various local dishes. Traditional use of the plant is cardiotonic, anthelmintic agent, etc. Previously we have found in our studies that essential oils isolated from the leaves of the plant exhibit anthelmintic and antihypertensive activities [2]. *C. bejolghota* bark extract was also reported for antimicrobialactivity [3 - 5]. However, the essential oil of the bark of the plant was not evaluated earlier as anthelmintic and antihypertensive agents.

Hypertension is a chronic disease worldwide with prognosis of elevated blood pressure, heart attack, kidney dysfunction, cardiac arrest and loss of life. An individual with systolic blood pressure above 140 mm (Hg) and diastolic blood pressure above 90 mm (Hg) is considered to be hypertensive [6 - 7]. The angiotensin-converting enzyme controls the fluid volume and imparts a major role in raising blood pressure. It converts inactivedecapeptide angiotensin I into the potent vasoconstrictor octapeptide angiotensin II, which is a crucial step in causing hypertension [8]. This enzyme can be inhibited by using synthetic angiotensin-converting enzyme (ACE) inhibitors which have many undesired toxic effects [9]. The presence of ACE inhibitory compounds has been detected in many essential oil bearing plants like *Periploca laevigata* and *Ajuga pseudoiva* [10 - 11]. Antioxidant deficiency leads to cardiac diseases [12]. The phytoconstituents present in essential oil contribute to the resistance to the oxidative damage of the cells, which results in cardio protective action [13]. Therefore, the current research is focused on natural antioxidants and ACE inhibitors from essential oil which may be considered as substitute therapy for high blood pressure.

Worm infection continues to be a major threat in the world. Countries in the tropical regions are largely affected by parasitic worms and are also major constraints for the management of livestock throughout the globe. School-going students are mainly infested with worm due to improper sanitary conditions. Currently available drugs have developed resistance to various types of worms, which warrants the development of newer drugs. Helminthiasis is controlled by the use of semi-synthetic drugs but due to unwanted side-effects and disadvantages such as high cost, drug resistance, and unavailability in developing countries, alternative studies are always needed [14 - 15]. Flatworm associated pulmonary arterial hypertension is a fatal complication of chronic worm infection and a leading cause of hypertension related mortality worldwide. In chronic form of infection flatworms lay eggs in the portal and mesenteric venous systems. An antigenic response is developed in the body due to the presence of eggs, leading to fibrotic lesions, granuloma and portal shunting. Portal shunting leads to portal hypertension. The chronic form of worm infection leads to hepatosplenic disease and pulmonary complications including pulmonary arterial hypertension [16].

The study on essential oils of various plantparts and their biological activities continues to be an important area of research and development [17 - 19]. In the continuation of our current research on the identification of bioactive substances from plants of North East India [4, 20 - 22], we have investigated the antioxidant, anti-hypertensive (angiotensin-converting enzyme method) and anthelmintic properties of the *C. bejolghota* bark essential oil and the results are found to be promising.

2. MATERIALS AND METHODS

2.1. Plant Material

The bark of the plants was gathered from Rowriah, Jorhat district of Assam in April 2014. The identification of the selected species was confirmed by Dr. A. A. Mao, Botanical Survey of India, (BSI) Shillong, Meghalaya, Indiaand a voucher specimen (Acc No: NPC/0322) was deposited at CSIR NEIST, Jorhat.

2.2. Determination of volatile components from *C. bejolghota* bark essential oil

2000 grams of bark was ground into coarse powder and hydro-distilled in a Clevenger apparatus for 6 h to obtain about 3.5 mL of essential oil. The oil was stored in a refrigerator at 4 ^{o}C and was then analyzed by gas chromatography and gas chromatography-mass spectroscopy technique (Trace, DSQ, Thermo Fisher Scientific, Austria) coupled with an FID detector. By injecting a homologous series of *n*-alkanes (C_2-C_{28}),the retention indices of the chemical components were determined and compared with those in the literature.

2.3. Gas chromatography/mass spectrometry (GC/MS)

The volatile components of the oil were identified by GC-MS using a Trace DSQ instrument from M/S Thermo Fisher Scientific. The capillary model is Thermo Fisher Scientific TR-5MS 30 m × 0.25 mm ID × 0.25 µm. The temperature of the injector and detector (FID) was kept at 280 °C. The machine was equipped with temperature controlling devices with initial temperature set at 50 ^{o}C for one min, maximum temperature 350 ^{o}C, ion source 200, split flow rate of 50 mL/min and split ratio 10:1; ultrapure helium was used as transporter gas witha flow rate of 30 mL/s. The mass spectra were acquired using a quadruple mass analyzer in the range of m/z 50 to m/z 450; with running time of 45 min. The retention indices of the constituents were estimated and related to n-alkanes [23]. Using the NIST library database accessible with the instrument along with Wiley library spectra and literature, the constituents were interpreted by relating their mass spectra and retention indices [24 - 25]. The percentage of each component of the oil was represented by relative peak area in the chromatogram and was obtained from electronic integration measurements using FID detection without correction of response factor.

2.4. Biological Activities of *C. bejolghota* bark essential oil

2.4.1. DPPH radical-scavenging activity determination

The radical-scavenging ability of *C. bejolghota*bark essential oil was measured using the DPPH method [26]. Different concentrations of essential oil (0.1 - 1.0 mg/mL) were added to 375 µL of methanol and 125 µL of DPPH solution. The absorbance of the tested compounds and blank was measured at 517 nm after 60 min of incubation. The study is compared with ascorbic acid which is taken as a standard. The absorbance reading of the reaction is inversely proportional to the free radical-scavenging activity. The percentage inhibitory percentage of the DPPH radical was estimated according to the following formula:

$$\text{\% of DPPH scavenging activity} = \frac{(A_o - A_t)}{A_o} \times 100\ \%$$

where, A_o and A_t represent the absorbance of the control reaction and *C. bejolghota* bark essential oil, respectively. IC_{50} value is the measure of the oil concentration (mg/ml) showing 50 % activity when tested *in-vitro*. All experiments were performed in triplicate.

2.4.2. β-Carotene bleaching by linoleic acid assay

The ability of the *C. bejolghota*bark essential oil to bleachβ-carotene was estimated using the method of Hajji *et al.* [10].Concentrated solution of β-carotene and linoleic acid was prepared by dissolving 0.5 mg of β-carotene and 25 µL of linoleic acid in 1 mL of chloroform. 200 µL of Tween 40 was added which served as an emulsifying agent. The chloroform was vaporized at 40 ^{o}C and mixed with 100 mLof distilled water. Then the reaction mixture was shaked properly. The essential oils with different concentrations were then mixed with 2.5 mL of the β-carotene and linoleic acid emulsion and incubated at 50 ^{o}C for 2 hours. The absorbance of the tested essential oil and standard Ascorbic acid was measured at 470 nm. All the experiments were performed in triplicate.

2.4.3. Ferric-reducing activity

By following the standard method of Hajji *et al.* the reducing power of the *C. bejolghota*bark essential oil was estimated [10]. The essential oils prepared in various doses were mixed with 1.25 mL of 0.2 M phosphate buffer (pH 6.6) and 1.25 mL of potassium ferricyanide solution (10 g/L) and incubated at 50 ^{o}C for half an hour. 1.25 mL of trichloroacetic acid (100 g/L) was added after incubation and the reaction mixture was centrifuged for 10 min at 1000 rpm. The supernatant (1.25 mL) from each sample mixture was collected and mixed with 0.25 mL of 1.0 g/L ferric chloride solution and 1.25 mL of distilled water in a sampler. After 10 min reaction, the absorbance was measured in UV at 700 nm. The absorbance of the reaction mixture is directly proportional to the reducing power. All the experiments were carried out in triplicate.

2.4.4. In vitro anti-hypertensive activity by ACE inhibitory method

The ACE inhibitory activityof *C. bejolghota*bark essential oil was assessed using the method reported by Nakamura *et al.* [27]. Accordingly, standard ACE inhibitory drug Ramipril was used for this assay. 200 µL of 5 mM hippuryl-l-histidyl-lleucine (HHL) was added to 80 µL of different doses (50, 100, 150, 200 µg/mL) of *C. bejolghota*bark essential oil and kept at 37 ^{o}C for 3 min. Borate buffer having pH 8.3 was used to prepare a solution of *C. bejolghota*bark essential oil and HHL. 20 µL of ACE was added to this solution which was then incubated at 37 ^{o}C for 30 min. The enzyme reaction was quenched by adding 0.1 M HCl to the mixture. The released hippuric acid was then extracted with 1.7 mL of ethyl acetate and redissolved in 1 mL of distilled water. The results were measured at 228 nm using a UV spectrophotometer. The average value was determined from triplicate studies. The activity was determined according to the following formula

$$\text{ACE inhibitory activity (\%)} = \frac{(A_o - A_t)}{A_o} \times 100\ \%$$

where, A_t is the measure of absorbance of the released hippuric acid when ACE inhibitor component is present; $A_o$is the absorbance of the released hippuric acid when ACE inhibitor component is absent.

2.4.5. Anthelmintic activity of C. bejolghota bark essential oil

2.4.5.1. Culture of Caenorhabditis elegans

Nematode growth medium (NGM) was used at 25 ^{o}C for the culture of *C. elegans.* The medium was consisted of 1.25 g of peptone, 1.5 g of NaCl, 8.5 g of agar, 0.25 % cholesterol, 0.5 mM $CaCl_2$, 0.5 mM $MgSO_4$, and 12.5 mM KH_2PO_4 in 500 mL of water. The strain *Escherichia*

coli OP50 was used as a nutrient for the survival of the nematode worm [28 - 29]. The standard drug albendazole was procured from Sigma-Aldrich. Albendazole was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 0.1 % and was used for the assay.

2.4.6. In vitro Assay for Caenorhabditis elegans

2.4.6.1. Assays using adults

C. elegans strain was cultured on petri-dishes containing NGM and *E. coli* OP50 strain was used as nutrient sources. After adding different doses of essential oil and albendazole, petri-dishes were incubated at 20 ^{o}C for three days. Then the survival of the nematodes was calculated. Three different experiments were performed independently.

2.4.6.2. Egg hatch assay

Sufficient amounts of nutrient growth medium containing adult *C. elegans* were taken and centrifuged at 600 rpm for 7 min. A lysing solution was used for the suspension of the pellet. Separated eggs were then taken in M9 buffer and then the numbers of eggs were calculated. The eggs were re-suspended in a 96-well plate with *E. coli* OP50 strain. The eggs were then treated and incubated with different doses of essential oil and albendazole at 20 ℃ for 15 hours. The percentage of hatched eggs and L1 larvae at different doses after drug treatment were counted at the end of the incubation. Experiments were performed in triplicate independently.

2.4.6.3. Larval development assay

Larvae of L1 stage were collected and taken in 96 well plates along with *E. coli* OP_{50} strain as nutrient. The plates were then treated with various doses of the essential oil and albendazole. The plates were maintained at 20 ℃ for 24 hours. The mortality percentage of larvae was calculated out in three different independent experiments.

2.4.6.4. ED_{50} calculation

The numbers of living adult and dead nematodes were calculated at the end of each experiment. Immotile nematodes with linear or spiral bodies were considered as dead and accordingly ED_{50} was calculated. ED_{50} is the concentration of a drug taken which is lethal against 50 % of the worms.

2.4.6.5. Light microscopy

Light microscopy was used to study the morphology *of C. elegans.* For this, nematodes were transferred from the petri-plates onto the glass slides and observed under an Olympus B_51 light microscope using brightfield or differential interference contrast (DIC). The images were captured using an Olympus DP12 digital camera.

2.4.7. Statistical analysis

All the results were statistically calculated and expressed as mean ± S.E.M. The software used was Graph Pad Prism version 8 (Graph Pad Software Inc., La Jolla, CA, USA). One-way Analysis of Variance (ANOVA) followed by Turkey tests was done to determine the difference between standard and tested dose groups. $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Chemical composition and yield of the essential oil

A total of twenty-eight components were identified from the essential oilof *C. bejolghota* bark accounting for 97.06 % of the total content identified (Figure 1, Figure S1 and Table 1). The yield of the essential oil is 0.810 %. Sesquiterpenes (48.21 %), oxygenated monoterpenes (35.09 %) and monoterpenes hydrocarbon (13.2 %) constitute the major compounds. The major compounds identified are α-terpineol (18.57 %), α-cubebene (12.75 %), linalool (7.76 %) and terpinen-4-ol (5.64 %). A few studies have been reported on essential oil of *C. bejolghota* bark earlier. The essential oil collected from Thailand showed the presence of 1,8-cineole (40.24 %) as the major constituent [30]. A previous study from the same species of India showed variation of the main component. In contrast to our study, 1,8-cineole (31.3 %) was reported as the major compound by Choudhury *et al.* [31]. Another study showed the presence of α-terpineol (18.20 %) and (*E*)-nerolidol (15.30 %) as the major compound [32]. However α-pinene,camphene, 3-carene, *p*-cymene, limonene, γ-terpinene,terpinolene,linalool oxide,*trans*-carveol, α-cubebene, α-copaene, β-caryophyllene, β-humulene,γ-muurolene,γ-gurjunene, α-patchoulene, β-cadinene,*cis*-calamenene, α-cadinene,α-calacorene, *cis*-α-copane-8-ol, α-cadinol, and aristolonewere absent in the study conducted by Baruah *et al.* [32].

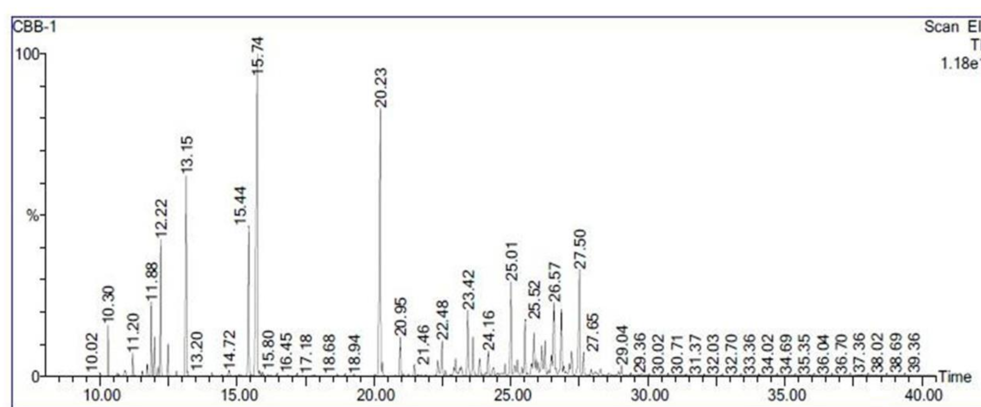

Figure 1. Chromatogram of essential oil of *C. bejolghota* bark.

Table 1. Chemical compounds identified by GC-MS from *C. bejolghata* bark essential oil.

Sl No	Constituent	[a]RT	[b](RI)	[c](RI)[lit]	Content %	[c]Identification	Molecular formula	Molecular weight	Ref
1	α-pinene (1)	10.299	937	939	1.27	RI, MS	$C_{10}H_{16}$	136.23	49
2	camphene (2)	10.905	951	953	0.98	RI, MS	$C_{10}H_{16}$	136.23	49
3	3-carene (3)	11.200	1011	1011	0.63	RI, MS	$C_{10}H_{16}$	136.23	49
4	*p*-cymene (4)	11.730	1024	1026	2.06	RI, MS	$C_{10}H_{14}$	134.22	49
5	1,8-cineole (5)	11.880	1026	1031	2.19	RI, MS	$C_{10}H_{18}$	154.25	25
6	limonene (6)	11.995	1029	1032	4.10	RI, MS	$C_{10}H_{16}$	136.00	50
7	γ-terpinene (7)	12.220	1062	1060	0.94	RI, MS	$C_{10}H_{16}$	136.23	25

8	terpinolene (8)	12.490	1065	1063	0.85	RI, MS	$C_{10}H_{16}$	136.23	50
9	linalool oxide (9)	12.795	1070	1074	0.28	RI, MS	$C_{10}H_{18}O_2$	170.30	51
10	linalool (10)	13.150	1084	1099	7.76	RI, MS	$C_{10}H_{18}O$	154.25	52
11	*trans*-carveol (11))	14.716	1178	1188	2.84	RI, MS	$C_{10}H_{16}O_2$	152.2	53
12	terpinen-4-ol (12)	15.436	1189	1191	5.64	RI, MS	$C_{10}H_{18}O$	154.25	54
13	α-terpineol (13)	15.741	1195	1197	18.57	RI, MS	$C_{10}H_{18}O$	154.25	55
14	α-cubebene (14)	20.233	1350	1351	12.75	RI, MS	$C_{15}H_{24}$	204.35	56
15	α-copaene (15)	21.464	1375	1377	3.86	RI, MS	$C_{15}H_{24}$	204.35	57
16	β-caryophyllene (16)	22.319	1411	1416	0.89	RI, MS	$C_{15}H_{24}$	204.35	23
17	α-caryophyllene (17)	22.484	1421	1418	0.64	RI, MS	$C_{15}H_{24}$	204.38	48
18	β-humulene (18)	23.420	1442	1439	2.05	RI, MS	$C_{15}H_{24}$	204.35	58
19	γ-muurolene (19)	23.610	1477	1477	2.53	RI, MS	$C_{15}H_{24}$	204.35	56
20	γ-gurjunene (20)	23.850	1476	1479	4.16	RI, MS	$C_{15}H_{24}$	204.35	59
21	α-patchoulene (21)	24.165	1489	1485	4.33	RI, MS	$C_{15}H_{24}$	204.35	60
22	β-cadinene (22)	25.515	1501	1518	4.73	RI, MS	$C_{15}H_{24}$	204.35	53
23	*cis*-calamenene (23)	26.246	1515	1521	1.59	RI, MS	$C_{15}H_{22}$	202.34	61
24	α-cadinene (24)	26.571	1525	1530	0.76	RI, MS	$C_{15}H_{22}$	204.35	62
25	α-calacorene (25)	27.646	1542	1546	0.99	RI, MS	$C_{15}H_{20}$	200.32	56
26	*cis*-α-copane-8 ol (26)	28.276	1621	1624	2.35	RI, MS	$C_{15}H_{24}O$	220.35	63
27	α-cadinol (27)	26.841	1652	1653	2.42	RI, MS	$C_{15}H_{26}O$	222.37	50
28	aristolone (28)	27.501	1759	1762	4.76	RI, MS	$C_{15}H_{22}O$	218.34	64
	Total				**97.06**				

[a]RT: Retention time, [b]RI: Retention indices determined on a Thermo Fisher Scientific TR-5MS 30m × 0.25 mm ID× 0.25μm with reference to n-alkanes, [C]RI: Retention indices from literature, [c]MS: Identification by GC-MS analysis, using NIST and Wiley library spectra and literature studies.

3.2. Antioxidant activity *in vitro* of *C. bejolghota* bark essential oil

3.2.1. DPPH radical-scavenging activity

The ability of 2,2-diphenyl-1-picrylhydrazyl free radical to accept an electron or hydrogen radical from antioxidants to form a steady diamagnetic molecule is the basis of DPPH radical scavenging activity. Free radical scavenging activities of *C. bejolghota* bark essential oil, estimated by the DPPH method are shown in Table 2. The essential oil has sufficient radical scavenging capacity with a percentage inhibition of 88.62 ± 0.64 % (IC_{50} = 0.561 ± 0.003 mg/mL) which is significantly comparable with ascorbic acid with 89.30 ± 0.05 % inhibition at a concentration of 1 mg/mL (IC_{50} = 0.103 ± 0.008 mg/mL).

Table 2. Antioxidant activity of standard and *C.bejolghata* bark essential oil by DPPH method.

Tested material	Tested concentration (mg/mL)	% inhibition ± SEM	IC_{50} (mg/mL)
C. bejolghota bark essential oil	0.1	5.38 ± 0.68	0.561 ± 0.003
	0.2	14.17 ± 0.14	
	0.4	40.95 ± 0.29	
	0.8	72.53 ± 0.37[a]	
	1	88.62 ± 0.64[b]	
Ascorbic acid	0.1	34.21 ± 0.14	0.103 ± 0.008
	0.2	66.86 ± 0.05	
	0.4	71.33 ± 0.05[a]	
	0.8	85.90 ± 0.11	
	1	89.30 ± 0.05[b]	

a,b: Means with the same letter within the same column are not significantly different at $p < 0.05$
95 % CL = confidence interval at 95 % confidence level
IC_{50} values are represented in % inhibition ± SD (SD = standard deviation (n = 3)).

3.2.2. β-Carotene bleaching by linoleic acid assay

The colour change of β-carotene is used to estimate the inhibition of oxidation activity of the new compound [33]. Antioxidant activity is directly proportional to the concentration of essential oil. At a concentration of 1 mg/mL, *C. bejolghota* bark essential oils hows significant inhibition of 89.25 ± 0.05 % which was as efficient as that of standard antioxidant ascorbic acid with 85.97 ± 0.11% inhibition. The IC_{50} was found to be 0.359 ± 0.007 mg/mL for *C. bejolghota*bark essential oilcompared to the IC_{50} of 0.295 ± 0.002 mg/mL exhibited by standard ascorbic acid (Table 3).

3.2.3. Ferric-reducing activity

The electron or hydrogen donating ability of antioxidants can be evaluated byreducing power assay method [33]. The reducing power of bioactive compounds can be directly correlated to antioxidant activity. The result is dependent on the absorbance which is directly proportional to the reducing power. The capability of the essential oiland standard ascorbic acid to reduce Fe^{3+} to $Fe^{2+}$was measured in this study [34]. The result showed that with increasing concentration, the ferric reducing activity of the essential oil and ascorbic acid was inclined. *C. bejolghota* bark essential oil showed an inhibition rate of89.21 ± 1.004 % which was significantly equivalent to that of standard antioxidant ascorbic acid with 90.14 ± 0.057 %

inhibition at the highest treated concentration of 1 mg/mL (Table 4). The IC_{50} was calculated and found to be 0.381 ± 0.032 mg/mL for *C. bejolghota* bark essential oil, as compared to the $IC_{50}$of 0.194 ± 0.004 mg/mL exhibited by standard ascorbic acid . Due to the presence of α-terpineol, α-cubebene, linalool and terpinen-4-ol in the oil, the reducing potential was enhanced. Antioxidant properties of α-terpineol, linalool and terpinen-4-ol were reported previously [35 - 37].

Table 3. Antioxidant activity of standard and *C. bejolghata* bark essential oil by β-Carotene method.

Tested material	Tested concentration (mg/mL)	% inhibition ± SEM	IC_{50} (mg/mL)
C. bejolghota bark essential oil	0.2	33.69 ± 0.61	0.359 ± 0.007
	0.4	53.77 ± 0.05	
	0.6	71.11 ± 0.23[a]	
	0.8	85.73 ± 0.06[b]	
	1	89.25 ± 0.06[c]	
Ascorbic acid	0.2	34.62 ± 0.05	0.295 ± 0.002
	0.4	66.70 ± 0.15	
	0.6	71.31 ± 0.05[a]	
	0.8	85.97 ± 0.11[b]	
	1	89.57 ± 0.29[c]	

a,b: Means with the same letter within the same column are not significantly different at $p < 0.05$
95 % CL = confidence interval at 95 % confidence level
IC_{50} values are represented in % inhibition ± SD (SD = standard deviation (n = 3)).

Table 4. Antioxidant activity of *C.bejolghata* bark essential oil by ferric-reducing method.

Tested material	Tested concentration (mg/mL)	% inhibition ± SEM	IC_{50} (mg/mL)
C. bejolghota bark essential oil	0.2	32.92 ± 3.64	0.381 ± 0.032
	0.4	50.26 ± 2.34[a]	
	0.6	71.39 ± 0.0	
	0.8	85.65 ± 0.05[b]	
	1	89.21 ± 1.00[c]	
Ascorbic acid	0.2	44.84 ± 0.26	0.194 ± 0.004
	0.4	65.81 ± 0.34[a]	
	0.6	75.55 ± 0.05	
	0.8	86.54 ± 0.17[b]	
	1	90.14 ± 0.057[c]	

a,b: Means with the same letter within the same column are not significantly different at $p < 0.05$
95 % CL = confidence interval at 95 % confidence level
IC_{50} values are represented in % inhibition ± SD (SD = standard deviation (n = 3)).

3.3. *In vitro* anti-hypertensive activity by ACE inhibitory method

Table 5. ACE inhibitory activity of Ramipril and *C.bejolghata* bark essential oil.

Tested material	Tested concentration (mg/mL)	% inhibition ± SEM	IC_{50} (µg/mL)
C. bejolghota bark essential oil	50	51.35 ± 0.19	33.43 ± 0.46
	100	75.42 ± 0.05	
	150	86.29 ± 0.22[a]	
	200	98.42 ± 0.15[b]	
	50	53.19 ± 0.11	
Ramipril	100	76.16 ± 0.20	27.69 ± 0.67
	150	86.57 ± 0.09[a]	
	250	98.86 ± 0.05[b]	
	50	51.35 ± 0.19	
	100	75.42 ± 0.05	

a,b: Means with the same letter within the same column are not significantly different at $p < 0.05$

95 % CL = confidence interval at 95 % confidence level

IC_{50} values are represented in % inhibition ± SD (SD = standard deviation (n = 3)).

The hindrance of ACE is regarded as one of the important curative approaches in the management of hypertension. The ACE inhibitory activity of *C. bejolghota* bark essential oilwas investigated using ramipril as a standard *C. bejolghota* bark essential oil showed dose-dependent *in vitro* antihypertensive activity. The essential oil showed 98.42 ± 0.14 % inhibition at 200 µg/mL (IC_{50} value of 33.43 ± 0.46 µg/mL) which was as efficient as that of the standard ACE inhibitor ramipril with 98.86 ± 0.05 % inhibition (IC_{50} value of 27.69 ± 0.67 µg/mL) ($p > 0.05$) (Table 5). From the result, it is seen that *C. bejolghota*bark essential oil can be implemented as angiotensin-converting enzyme inhibitor for the prevention and cure of hypertension. Many studies have been performed on the ACE activity of essential oils. From the published reports , it was found that *A. pseudoiva*and *T. algeriensis*essential oils showed a dose-dependent angiotensin-converting enzyme inhibitory activity. These plants showed 50 % inhibition at a value of 65.5 µg/mL and 150 µg/mL, respectively [38].

The major compounds present in the essential oilof *C. bejolghota* bark may be responsible for thesignificant antihypertensive activity. Monoterpene linalool (100 mg/kg/day) significantly reducedblood pressure and prevented cardiac hypertrophy in spontaneously hypertensive rats. The treated rats had an increased level of cytokine (IL-10) vasodilatation [39]. Linalool (200 mg/kg/day) reduced blood pressure in hypertensive rats due to vascular smooth muscle induced vasodilation [40]. Another study reported that, by modulating guanylyl cyclase and K+ channels, linalool induced endothelium-dependent vasorelaxation in mice [41]. The compounds α-terpineol and terpinen-4-ol were also reported to have antihypertensive property due to a decrease in peripheral vascular resistance [13, 42].

3.4. Anthelmintic activity

Caenorhabditis elegans is a free-living nematodewhich offers a suitable alternative model for the study of newer anthelmintic compounds. The adult nematodeis about 1 mm in length and can be easily cultured in a suitable laboratory environment. Thenematode grows naturally in moist soils of the temperate zone. This model organism is non-parasitic, lab friendly, economical and easily accessible, which makes it an excellent system for anthelmintic research [43 - 44].

3.5. Effect of essential oil on various life cycles of *C. elegans*

Table 6. The effect of *C. bejolghota*essential oil and albendazole on adult *C.elegans*.

Tested material	Tested concentration (mg/mL)	% inhibition ± SEM	ED_{50} (μg/mL)
C. bejolghota bark essential oil	50	28.88 ± 2.22	80.53 ± 3.57
	100	73.33 ± 3.84[a]	
	250	84.44 ± 2.22[b]	
Albendazole	50	44.44 ± 2.22	46.62 ± 3.42
	100	68.88 ± 2.22[a]	
	250	88.88 ± 2.22[b]	

a,b: Means with the same letter within the same column are not significantly different at $p < 0.05$

95 % CL = confidence interval at 95 % confidence level

ED_{50} values are represented in % inhibition ± SEM (SEM = Standard error of mean (n = 3)).

Table 7. The effect of *C. bejolghota* essential oil and albendazole on the egg hatching of *C. elegans*.

Tested material	Tested concentration (mg/mL)	% inhibition ± SEM	ED_{50} (μg/mL)
C. bejolghota bark essential oil	50	41.33 ± 1.76	68.27 ± 3.51
	100	65.33 ± 0.67[a]	
	150	80.66 ± 1.33[b]	
Albendazole	50	55.33 ± 0.67	42.34 ± 1.84
	100	62.66 ± 0.67[a]	
	150	86.66 ± 0.67[b]	

a,b: Means with the same letter within the same column are not significantly different at $p < 0.05$

95% CL = confidence interval at 95 % confidence level

ED_{50} values are represented in % inhibition ± SEM (SEM = Standard error of mean (n = 3)).

The therapeutic potency of *C. bejolghota* bark essential oil on *C. elegans* shows that the survival ability of nematodes is directly proportional to the oil concentration used. Three-day treatment showed that the *C. bejolghota* bark essential oilhad prominent activity against adult nematode resulting in 84.44 ± 2.22 % worm mortality at 250 μg/mL (ED_{50} = 80.53 ± 3.57 μg/mL) which was significantly similar to the positive control albendazole with an inhibitory

effect of 88.88 ± 2.22 % (P>0.05). The ED_{50} of albendazole was 46.62 ± 3.42 µg/mL and at 250 µg/mL less than 16 % of the worms remained viable (Table 6).

The treatment of the eggs of *C. elegans* with150 µg/mL of *C. bejolghota* bark essential oilresulted in an ovicidal activity of 80.66 ± 1.33 % (ED_{50} = 68.27 ± 3.51µg/mL) which was significantly similar to the positive control albendazole with an egg hatch inhibition rate of 86.66 ± 0.67 % (ED_{50} = 42.34 ± 1.84 µg/mL) ($P > 0.05$). At essential oil concentrations above 150 µg/mL, the hatching of eggs decreased remarkably (Table 7). The egghatching rate decreased with increasing essential oil concentration. Moreover, *C. bejolghota* bark essential oil showed potent anthelmintic activity by making the eggs unviable and preventing their hatching and development into larvae.

Table 8. The effect of *C. bejolghota*essential oil and albendazole on larval development assay.

Tested material	Tested concentration (mg/mL)	% inhibition ± SEM	ED_{50} (µg/mL)
C. bejolghota bark essential oil	50	37.33 ± 0.67	72.40 ± 2.68
	100	68.00 ± 2.00[a]	
	150	80.00 ± 1.15[b]	
Albendazole	50	52.66 ± 0.67	37.74 ± 2.24
	100	68.66 ± 0.67[a]	
	150	82.66 ± 0.67[b]	

a,b: Means with the same letter within the same column are not significantly different at $p < 0.05$

95 % CL = confidence interval at 95 % confidence level

ED_{50} values are represented in % inhibition ± SEM (SEM = Standard error of mean (n = 3)).

The larval dependent assay showed that larvae were more sensitive to various concentrations of drug than adult. Significant larval development activity was shown by *C. bejolghota* bark barkessential oil with 80.00 ± 1.15 % inhibition which was significantly similar toalbendazole with 82.66 ± 0.67 % inhibition at the highest treated concentration of 150 µg/mL. From the experiment, it can be interpreted that at a concentration of 37.74 ± 2.24 µg/mL and 72.40 ± 2.68 µg/mL, the survival of about 50 % of larvae was observed for albendazole and *C. bejolghota*bark essential oil, respectively. Thus, the treatment prevents the successful development of larvae into adult (Table 8, Table 9).

From a chemical point of view, the major compounds found in the essential oil have shown anthelmintic activity in previous studies. In recent years, terpenoids have been studied for their anthelmintic activity. The presence of α-terpineol and 1, 8-cineole may be responsible for the anthelmintic activity of *C. bejolghota* bark essential oil as these compounds present in *Callistemon citrinus*have significant anthelmintic activity [45]. Another major compound linalool (LC_{50} = 0.29 mg/mL) showed anthelmintic activity against *H. contortus* [46]. Terpinen-4-ol present in *Melaleuca alternifolia* was reported to have anthelmintic activity against *H. Contortus* [47]. The compound showed a parasite recovery rate of 43.18 %. In another study by Fernandez *et al.*, Terpinen-4-ol,1, 8-cineole and linalool were found to present larvicidal activity against *R. microplus* larvae [48].

Table 9. IC_{50} values of antioxidant, ACE inhibitory and anthelmintic activity of standard and *C. bejolghata* bark essential oil.

Test		IC_{50}	
		Ascorbic acid (mg/mL)	*C. bejolghota* bark essential oil(mg/mL)
Antioxidant activity	DPPH	0.103 ± 0.008	0.561 ± 0.003
	ß-Carotene	0.295 ± 0.002	0.359 ± 0.007
	Ferric-reducing	0.194 ± 0.004	0.381 ± 0.032
ACE inhibitory activity		Ramipril (µg/mL)	*C.bejolghata* bark essential oil (µg/mL)
		27.69 ± 0.67	33.43 ± 0.46
		ED_{50}	
Anthelmintic activity	Assay on different life stages of *C.elegans*	Albendazole (µg/mL)	*C. bejolghota* bark essential oil (µg/mL)
	Adult mortality assay	46.62 ± 3.42	80.53 ± 3.57
	Egg hatching assay	42.34 ± 1.84	68.27 ± 3.51
	Larval development assay	37.74 ± 2.24	72.40 ± 2.68

IC_{50} values are represented in % inhibition ± SD (SD = standard deviation (n = 3))
ED_{50} values are represented in % inhibition ± SEM (SEM = Standard error of mean (n = 3)).

3.6. Effects of essential oil on the morphology of *C. elegans* at different stages

The morphological features in the drug treated and control adult helminthes were studied through light microscopic analysis. Untreated worms showed no change in morphology or showed normal morphology and intact body with anterior region (AR) and posterior region (PR). The presence of a cuticular surface with a typical cephalic end along the body was found in control specimens (Figures 2A, 2C, 2E, 2G). After treating with albendazole and *C. bejolghota* bark essential oil, changes in morphological features were distinctively observed. Adults treated with *C. bejolghota* bark essential oil at a concentration of 50 µg/mL had disorganization in the posterior region (Figure 2B), which was also observed in nematode treated with 250 µg/mL *C. bejolghota* bark essential oil with damage and detached cuticle in the anterior region (Figure 2D). Nematode treated with 50 µg/mL albendazole had disorganized posterior region and developed an irregular pattern of cuticle striation. In many parts of the body cuticular surface was desquamated (Figure 2F). After being treated with 250 µg/mL albendazole, nematodes were twisted, crumbled and had their body pattern destroyed (Figure 2H). *C. bejolghota* bark essential oil induced the disruption of adult nematodes and altered the morphology of the cuticular surface due to the chemical constituents present in the essential oil. The modification was found in three-quarters of the helminths analyzed.

Disorganization of external structures in treated larvae was observed when compared with control organisms. The larvae from hatched eggs had a typical cylindrical body in the anterior region (AR) and posterior region (PR) (Figures 2I, 2K, 2M, 2O). Larvae treated with 50 µg/mL *C. bejolghota* bark essential oilhad lesion and desquamation in the cuticles (Figure 2J). Larvae

treated with 150 µg/ *C. bejolghota* bark essential oilhad a disorganized body structure and partially detached cuticle from the hypodermis was seen (Figure 2L). Larvae treated with 50 µg/mL albendazole had detached cuticle and lesions on the upper body surface (Figure 2N). Albendazole at a concentration of 150 µg/mL caused disorganized and fragmented body pattern (Figure 2P).

Prior to treatment, eggshad a transparent eggshell surrounding the embryo (Figures 2Q, 2S, 2U, 2W). Eggs treated with 50 µg/mL *C. bejolghota*barkessential oil showed a disorganized mass instead of the formation of larval stage (Figure 2R). Eggs treated with 150 µg/mL of *C. bejolghota* bark essential oil appeared granular, crumbly and had distorted ultrastructure (Figure 2T). Eggs treated with 50 µg/mL albendazole developed a disorganized pile of cells and appeared refractile (Figure 2V). Eggshells treated with 150 µg/mL albendazole weredigested and the egg surface was found to be broken (Figure 2X). Nearly 90 % of the treated eggs showed ultrastructural damages when analyzed Meaning thetreated eggs had severe damage in ultrastructure. Thus, the oil was highly effective in preventing maturation and hatching leading to embryo degeneration.

Although *C. bejolghota* is traditionally used as anthelmintic and cardiotonic agent in Assam,no scientific report is available to justify this traditional use.The results found in our study suggest that *C. bejolghota* bark can be used as a preventive and remedial measure to treat high blood pressure. *C. bejolghota* bark oil also effectively prevents helminthiasis due to adult mortality, interfering the egg hatching and larval development of *C. elegans*. However, different ED_{50} valueswere observed in the assays because of the sensitivity of various components of the essential oil towards different stages of the life cycle of *C. elegans*.

Our study shows that terpenoids are the major phytochemical constituents of the bark of *C. bejolghota*. Thus, these terpenoids are probably responsible for the antihypertensive and anthelmintic activity of the essential oil from this *C. bejolghota* bark. All these findings suggest that essential oil from the bark of *C. bejolghota* offers an alternative source for the control of hypertension and helminthic infections.

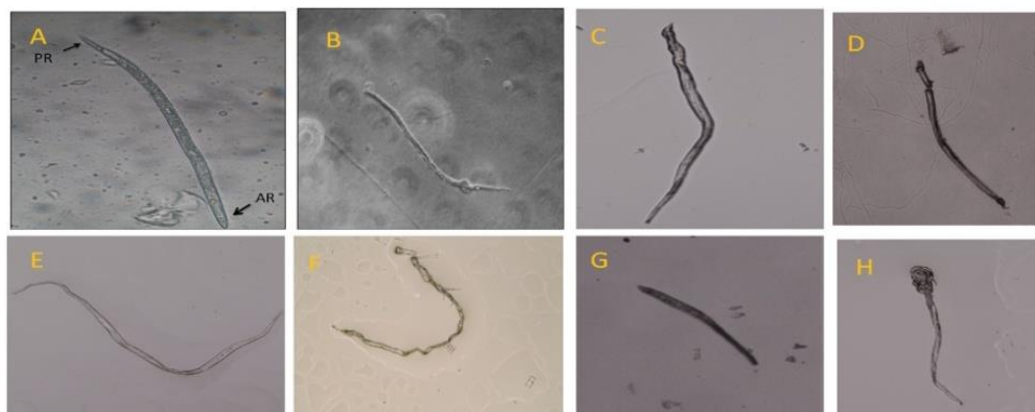

Figure 2. Morphology of different life stages of *C.elegans* by light microscopy. A: Untreated adults show typical morphology. AR, anterior region; PR, posterior region; B: Adults treated with 50 µg/mL *C. bejolghota* bark essential oil have disorganized posterior region; C: Untreated adult shows an intact body; D: Adults treated with 250 µg/mL *C. bejolghota* bark essential oil have damaged and detached cuticle at anterior region; E: Untreated adults show typical body structure; F: Adults treated with 50 µg/mL albendazole have disorganized posterior region and have irregular cuticle striation pattern; G: Untreated adults show typical morphology; H: Adults treated with 250 µg/mL albendazole have destroyed body and the worms were twisted;

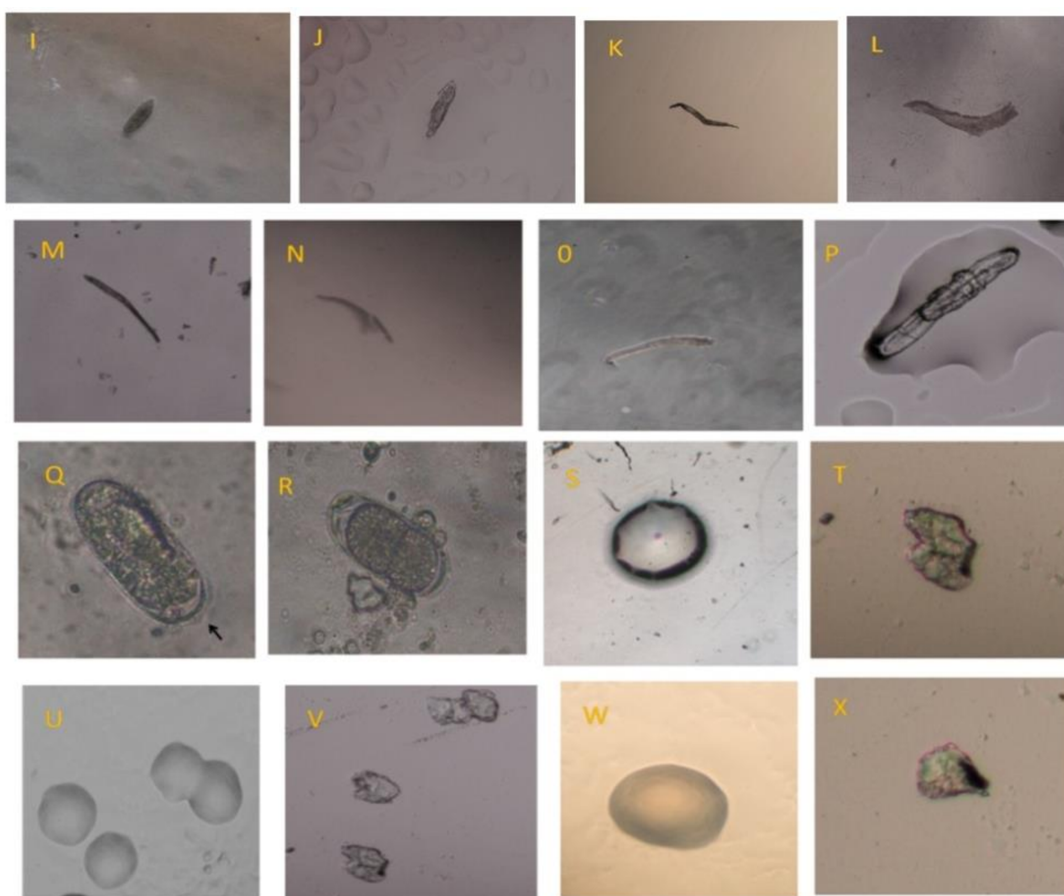

Figure 2. (continue) I: Untreated L1 larvae show an intact body with cylindrical architecture; J: L1 larvae treated with 50 µg/mL *C. bejolghota* bark essential oil have lesion in cuticles;K: Untreated L1 larvae show normal morphology with anterior and posterior region; L: L1 larvae treated with 150 µg/mL *C. bejolghota* bark essential oil have severe disorganized body structure; M: Untreated L1 larvae show normal body pattern; N: L1 treated with 50 µg/mL albendazole presented detached cuticle; O: Untreated larvae show typical morphology; P: L1 treated with 150 µg/mL albendazole have disorganized and fragmented body pattern. Q: Photograph of an egg prior to treatment. The arrow points to the transparent eggshell that surrounds the embryo; R: Egg treated with 50 µg/mL *C. bejolghota* bark essential oil showing a disorganized mass instead of the formation of L1 stage; S:Untreated egg; T: An egg treated with 250 µg/mL *C. bejolghota* bark essential oilappears granular and crumbly; U: Untreated egg; V: Egg treated with 50 µg/mLalbendazole developed unorganized pile of cells and appeared refractile; W:Untreated egg; X: The shell of egg treated with 250 µg/mLalbendazole is digested and disruption of egg surface is seen.

4. CONCLUSIONS

The present work deals with the development of affordable health care agents from ethnomedicinal odoriferous plants of the North East Indian flora. An exhaustive literature survey has shown that this is the first report on anthelmintic and antihypertensive properties of the essential oil of *C. bejolghota* bark. Our results demonstrate that *C. bejolghota* bark essential oil is capable of inhibiting angiotensin-converting enzyme with IC_{50} = 33.43 ± 0.46 µg/mL (IC_{50} of ramipril 27.69 ± 0.67 µg/mL). In addition, the essential oil has shown significant anthelmintic

property against *C. elegans* with an ED_{50} of 80.53 ± 3.57 μg/mL (46.62 ± 3.42 μg/mL for albendazole) in adult mortality assay. The larval development assay showed an ED_{50} of 72.40 ± 2.68 μg/mL (37.74 ± 2.24 μg/mL for albendazole) against *C. elegans* larva. Further, the egg hatching assay with this essential oil showed an ED_{50} of 68.27 ± 3.51 μg/mL (42.34 ± 1.84 μg/mL for albendazole).Thus, *C. bejolghota* bark essential oil could affect the growth of nematode, disrupt the ultrastructure of eggs and prevent the hatching of the eggs to develop into the larval stage. Among the 28 constituents, α-terpineol (18.57 %) was found to be the major component of *C. bejolghota* bark essential oil, followed by α-cubebene (12.75 %), linalool (7.76 %), and terpinen-4-ol (5.64 %). Thus, the knowledge of chemical constituents and bio-efficacy of *C. bejolghota* bark essential oil has demonstrated that this essential oil has excellent potential for its commercial and industrial applicationsagainst hypertension and helminthic infections.

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CRediT authorship contribution statement. Manobjyoti Bordoloi developed concept, designed the experiments and supervised all the experimental works, Antioxidant, Anthelmintic and ACE inhibition activities, chemical compounds from GC-MS analysis and preparation & finalization of the manuscript. Barnali Gogoi worked for the collection of plant material, isolation of essential oil, bioactivities of the samples and preparation of the manuscript. Kashyap J. Tamuli did the GC-MS data analysis and preparation of the manuscript. Snigdha Saikia and Neipihoi contributed to the data analysis and bioactivities of the essential oil. Hemanta K. Sharma contributed to the writingof the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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