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LEAVES AND BARK VOLATILE COMPOSITION AND THEIR ANTIOXIDANT ACTIVITY OF *CRYPTOCARYA STOCKSII* MEISN. -A VULNERABLE TREE GROWING IN INDIA

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Abstract

Cryptocarya stocksii Meisn. vulnerable evergreen tree grows in the Western Ghats of India. We investigated the chemical composition and antioxidant activity of C.stocksii *leaves and bark oil in terms of ferric reducing antioxidant power (FRAP) assay, scavenging of hydrogen peroxide (H_2O_2), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and inhibition of lipid peroxidation. GC-MS analysis of essential oil of leaves revealed the presence 43 compounds were identified in collective essential oil and 32 compounds in bark collective essential oil. Leaves oils were characterized by Nerolidol (23.09%), α-Pinene (12.58%), 3-Carene (11.5 %) and Camphene (8.14%), as the major compounds. The main compounds of Bark oil were Longifolene (54.4%), Carvacrol (29.6%), Nerolidol (2.5%) and Borneol (1.6%).The results showed that antioxidant activity of* C. stocksii *leaves and Bark oil was dose dependent and increased with increasing the essential oil concentration. The study concludes that C.* stockii *leaves and Bark contain terpenoid rich oil exhibiting antioxidant activity. This is the first paper reporting the antioxidant activities and chemical composition of the essential oils of this species.*

Keywords: *Cryptocarya stocksii, Lauraceae, essential oils, Antioxidant activity, Longifolene.*

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1.INTRODUCTION:

Essential oil-bearing plants have been collected from the cultivated and wild since prehistory for their flavor and fragrance properties, and the herbs, spices and related aromatic plants were valuable commodities for virtually all ancient civilizations [1,2] .Essential oils are complex volatile compounds produced in different plant parts which are known to have various functions in plants. The complexity in essential oil is due to terpene hydrocarbons as well as their oxygenated derivatives such as aldehydes, ketones, alcohols acids and esters. Essential oils from medicinal and aromatic plants have been known to possess biological activity, notably antioxidant activities, antibacterial and antifungal [3, 4]. Biological activity of essential oils depends on their chemical composition determined by genotype and influenced by environmental and agronomic conditions [5,6].In recent years, the essential oils have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds [7-8]. Essential oils are rich in phenolic compounds, and for this main reason, attract investigators to evaluate their activity as antioxidants or free radical scavengers. The essential oils of basil, cinnamon, clove, nutmeg, oregano and thyme have proven radical-scavenging and antioxidant properties [9]. Efforts have also been made to explore the potential of some essential oils for the treatment of infectious diseases in order to substitute standard pharmaceutical remedies [10].

Lauraceae is a large family of woody plants composed of approximately 2,750 species, distributed in 54 genera distributed throughout tropical to subtropical latitudes. The species are predominantly trees and mostly aromatic in nature [11, 12]. Most of the Lauraceae family members are characterized by a woody habit and are of great economic importance worldwide, as they provide valuable timber, aromatic oils and important substances that are widely employed in the pharmaceutical and food industries [13]. Recent studies showed that many species of the Lauraceae members were found to exhibit useful biological activities such as antitumour, antispasmodic, antipyretic, anticonvulsant, antibacterial, fungicidal, cytotoxic, and cruzain inhibitory activities and antiviral [14]. They contained interesting group of natural products, such as alkaloids, flavonoids, monoterpenes and sesquiterpenes, triterpenes and sterols, 2-pyrones, benzophenones and arylpropanoids[15]. In addition, compounds from the medicinal plants of this family have been found to show antioxidant activities, which can promote anti proliferation of the human cancer cells [16, 17].

The genus *Cryptocarya* comprises about 350 species, most of which are confined to tropical Asia [18, 19].In the Indian subcontinent, the genus is represented by 15 species, of which 6 are known to be distributed in the Western Ghats of southern India [19].

Cryptocarya stocksii Meisn. is a medium sized, evergreen tree with a dense spreading canopy and distributed in the evergreen forests of Western Ghats of Karnataka, India. This is common along the streams and the current threat status as endemic and rare threatened tree at local level [20] and vulnerable at global level (IUCN, 1998) [21].

In spite of the relatively large number of *Cryptocarya* species known, little work on the essential oils of the genus has been reported. *Cryptocarya* genus have oil glands in their leaves, few species contains oil glands in bark and wood [22, 23]. Analysis of the leaves oil from *C. cunninghamii* showed the presence of bicyclogermacrene, benzyl benzoate (80.2%), β -phellandrene (11.8%), viridiflorene (9.1%), phenylethyl benzoate (1%) and methyl benzoate (trace). There were lesser amounts of the two furanoid linalool oxides (4.1 and 3.6%, respectively) and linalool (4.5%). Very less amounts of caryophyllene oxide and spathulenol (1.3 and 1.2%, respectively) were also present [24].

Phytochemical investigations of the essential oils of *C. mandioccana*, leaves oil yielded 64 compounds with predominance of δ -cadinene, β-caryophyllene, spathulenol, benzaldehyde caryophyllene oxide, β germacrene-D, and bicyclogermacrene [25]. Naves *et al.* investigated the leaves oil of *C. moschata* and *C. aschersoniana*, *C. aschersoniana* has been investigated and found to oil contained myrcene, 1,8-cineole, (+)-linalool and the two stereoisomeric linalool oxides, while *C. moschatum* contained (S)-linalool as its major constituent. [26].

Analysis of the essential oil from the *Cryptocarya* species, endemic to the Brazilian Atlantic rainforests, were examined by Telascrea *et al*. [27] shows monoterpene oil with linalool, α-terpinene and γ-terpinene as major compounds in *C. moschata*. Leaves oil of *C. botethensis* gave α-pinene, β-pinene and trans-verbenol were the major, components, while in *C. saligna* germacrene-D, bicyclogermacrene and spathulenol predominated. The component that is most commonly associated with *Cryptocarya* is that derived from *C. massoia*

bark, viz. massoia lactone, which has a pleasant peach smell. This compound (combined with its 6-heptyl- homologue) has previously has been obtained from the bark and heartwood of this species [28-30]. *C. cocosoides* produced oil in which bicyclogermacrene (3-26%), spathulenol (16-47%), massoia lactone (11-15%), and benzyl benzoate (0.2-5%) were the principal components. *C. bellendenkerana* gave leaves oil in which the major components were the terpenes viridiflorene (9.1%) limonene (8.3%) and β-phellandrene (11.8%). The principal components of the leaves oil of *C. lividula* were spathulenol (21.1%), bicyclogermacrene (26.1%), and β-eudesmol (6.1%). acetophenone and Benzaldehyde and were both present in amounts of less than 0.7%.[31].

In recent study 71 were identified in the essential oil from leaves of *C. alba* composed mainly of (E)-βbergamotene , viridiflorol and germacrene-D, sabinene , camphene ,β-eudesmol , eucalyptol , terpin-4-ol, p-cymol, cineol, α-pinene, β-pinene and borneol-terpineol [32].Further, phytochemical analysis of *C. stocksii* bark extracts revealed the presence of significant levels of alkaloids, flavonoids and moderate amounts of steroids, and phenols. It exhibited moderate to strong antioxidant activity in terms of FRAP, DPPH and analgesic activity [33, 34]. Hence, considering that there were no reports in the literature of research carried out with this species, this present work aimed to establish for the first time, the chemical composition and their antioxidant activities of the leaves and bark essential oil of *C stocksii* grown in the South India.

2. MATERIALS AND METHODS:

2.1. Isolation of essential oil

Fresh leaves and bark (200 g) were collected from Western Ghats region of Karnataka, India. A voucher specimen (PS175/30.2014) was deposited at the Bioscience Department in P.G.Centre, Hemagangothri, Hassan. The fresh leaves and bark was cut into small pieces subjected to hydrodistillation for 4 h in a Clevenger-type apparatus. [35] The oil was dried over anhydrous sodium sulfate. The oil was stored at 4 °C in a refrigerator until the analysis by GC-MS. The yields (w/w) were calculated according to the weight of fresh plant material.

2.2. GC-MS analysis

The essential oils of *Cryptocarya stocksii* were analyzed on an Aligent gas chromatograph model 7890A, coupled to Agilent MS model 5975C MSD with data analysis and AMDIS software along with Quadrupole mass analyzer and electron impact ionization . GC equipped with a DB5 MS Column (30mL X 0.25mm ID X 0.25 µl thickness), programming from 50^0C (1min) to 300^0C at 10^0C/min, 5 min hold time. Helium as carrier gas at a flow rate 1.0 ml/min. Samples (1 µl) were injected manually in the split mode (10:1). Mass spectra data were acquired in the scan mode in m/z range 30-600. The components were identified based on their retention time, abundance and fragmentation patterns by reference to the NIST 2011 library. The percentages of each component are reported as raw percentages based on total ion current without standardization.

2.3. Antioxidant activity of the essential oil isolated from *Cryptocarya stocksii*

2.3.1. Anti-lipid peroxidation activity

Anti-lipid peroxidation activities of essential oil were estimated by TBARS method [36]. 0.5 ml of egg homogenate and essential oil with different concentrations (1-50µl) were made up to 1 ml with distilled water. 100µl of 0.07M $FeSO_4$ was added and incubated for 30 min at room temperature. To all test tubes 1.5 ml of acetic acid, 1.5 ml of TBA and 50µl of TCA were added. Vortexed and kept in boiling water bath for 1 hour. By adding 5 ml of butanol, test tubes were centrifuged at 3000 rpm for 10 mins. Absorbance of supernatant was measured at 530 nm. α-lipoic acid standard was used for comparison. The percent inhibition activity was calculated using the formula:

% Anti lipid peroxidation= (1−Extract absorbance) ⁄ (Control absorbance) ×100

2.3.2. DPPH scavenging activity

Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl radical method

Antioxidant activity of essential oil was determined using DPPH according to Giresha *et al.* [37]. The essential oil were taken at different concentration (1-50µl) and mixed with 5 ml of 0.1 mM methanolic solution of DPPH˙ and incubated at 20°C for 20 min in darkness. The control was prepared as above without any oil and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Ascorbic acid standard was used for comparison. Radical scavenging activity was expressed as percentage activity using the following formula.

Percentage radical scavenging activity = ([control absorbance − sample absorbance] / [control absorbance]) × 100.

2.3.3. *Hydrogen Peroxide Assay*

Hydroxyl radical-scavenging by phenanthroline - Fe (II) oxidation assay

The Fenton reaction is a key reaction in organisms which produces hydroxyl radicals. Hydroxyl radicals can attack aromatic compound hydroxylation to form hydroxylation products, which can be detected by colorimetric methods. The adding of antioxidants can reduce hydroxyl radicals and hydroxylation so that the efficiency of scavenging hydroxyl radical can be determined. The ability of the hydroxyl radical-scavenging was carried out as described previously [38]. Briefly, 600 µl of (5 mM) phenanthroline, 600 µl (5 mM) $FeSo_4$, 600 µl of EDTA (15 mM), 400 µl phosphate buffer (0.2 M, pH =7.4) and 800 µl (0.01%) H_2O_2 were added into 10-100 µl various concentration of the oil. After 1h of incubation at 37°C, the absorbance at 536 nm was recorded. Quercetin was used as standard.

The percentage hydrogen peroxide scavenging activity of samples was calculated as follows: Scavenged % Hydrogen peroxide = $(A_0 - A_1 / A_0) \times 100$

Here, A_0 was the absorbance of the control and A_1 was the absorbance of the samples.

2.3.4. Reducing power assay

Determination of reducing power

The reducing powers of extracts were determined according to Giresha *et al*., [37]. The essential oil were taken at different concentration (1-50µl) of essential oil and mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50°C for 20 min. The reaction was terminated by adding 2.5 ml of 10% TCA and the mixture was centrifuged at 3500 rpm for 10 min. An aliquot of supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride to form a colored complex which is measured at 700 nm against blank. The reducing power ability of the sample is determined by increase in absorbance of the sample. Butylated hydroxyanisole was used as standard for comparison.

3. Statistical analysis.

The experimental results were performed in triplicate. The results were analyzed by the Student t-test ($P < 0.05$) and experimental results were expressed as Mean ± Standard error (SE) for each experiments. The regression curve analysis was used to calculate IC_{50} using Graph Pad Prism version 5.02 statistical software. IC_{50} value is a concentration of essential oil required to scavenge 50% free radical and is inversely proportional to the activity of the essential oil.

4. RESULTS AND DISCUSSION:

4.1. Chemical constituents of the oil

The present study is the first report on the essential oil composition from leaves and bark of *C. stocksii* constituent primarily Terpenes, Hydrocarbons and Oxygenated compounds (Table 1).

The hydrodistillation of the leaves of *C.stocksii* yield clear colorless oil (0.1%) and bark gave pale yellow colored oil (0.2%). The GC-MS analyses of the leaves and bark oil revealed the presence of 43 and 32 components respectively. Comparative data on the different volatile oil constituents in the leaves and bark of *C.stocksii* is presented in Table 2. Each of the oils had characteristic aroma and the chromatograms of these essential oils are shown in Figures 1.

The chemicals analysis of the leaves and bark oil showed that the major compounds of terpenes and sesquiterpenes. In the leaves oil, most abundant compound was Nerolidol (23.09%). Other important compounds were α-Pinene (12.58%), 3-Carene (11.5 %) and Camphene (8.14%). The oil of bark (32 identified components) contained were Longifolene (54.4%), Carvacrol (29.6%), Nerolidol (2.5%) and Borneol (1.6%) as major components.

However, the chemical composition of the leaves and bark contain Monoterpenoid, Sesquiterpenoids , Hydrocarbons and Oxygenated compounds almost the same, whereas the percentages of Oxygenated compounds in the oils varied from leaves and bark (37.19%) and (21.87%). The Oxygenated compounds, and to a lesser extent the monoterpenes, are the major components of this essential oils. The concentration of Nerolidol was significantly lower in the bark oil (2.5%) than in the oils obtained from leaves (23.09%). Sesquiterpene nerolidol, also known as peruviol, is a naturally occurring sesquiterpene alcohol present in the essential oil of various plants with a floral odour [39, 40]. Longifolene (54.4%) and Carvacrol (29.6%) was one of the most abundant compounds in the oil from bark, but was less Longifolene (2.30%) and Carvacrol (0.04%) in leaves. Longifolene, a naturally occurring tricyclic sesquiterpene, has extensive applications in many different fields. Longifolene itself is used in the perfumery industry owing to its special woody fragrances [41]. Carvacrol is a monoterpenic phenol .It is known to have some pharmacological properties such as anti-inflammatory [42], antioxidant [43], antitumor [44], and antimicrobial [45] activity. The chemical composition observed in the present study was similar to the chemical composition already described in the literature for other species belonging to the same genus [31].

Monoterpenes are the leading contributor of most vital essential oils in nature. Since the monoterpenes are small molecules with two isoprene units, such as Camphene, 3-Carene, α-Pinene and β-Pinene and they are lipophilic; they are promptly consumed through the skin. Synthetic compounds can be used to break down the problems come across with herbal products by creating actions for the construction of such molecules, regardless of the original species. Indeed, ways have been developed for most of the natural molecules, but, given their commonly complex spatial arrangements, the industrial production is not practicable for the majority of examples [46, 47, 48, 49]

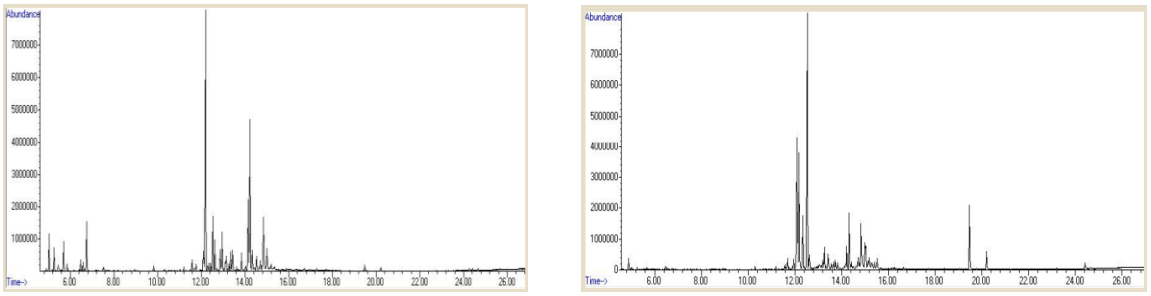
Table .1 Major chemical groups of essential oil composition of leaves and bark of *C.stocksii*

Major groups of oil components	Leaves	Bark
	Relative amount (%)	
Monoterpenoid	23.25	28.125
Sesquiterpenoids	2.32	3.125
Hydrocarbons	20.9	34.375
Oxygenated compounds	37.19	21.875
Others	16.45	28.125

Table.2- Percentage composition of the essential oils isolated from leaves and bark of *C.stocksii*

			Composition (%)	
Sl.No	**Retention Time**	**Compound name**	**Leaves**	**Bark**
1	5.0108	α-Pinene	12.58	0.8
2	5.1763	Methyl Isobutyl Ketone	0.47	-
3	5.2435	Camphene	8.14	0.8
4	5.4409	Benzaldehyde	3.12	-
5	5.6721	β-Pinene	10.3	0.89
6	5.9449	1,3,5-trimethylbenzene	-	0.03
7	5.9867	Decane	-	0.09
8	6.1352	4-Methyldecane	0.01	0.05
9	6.4148	β-Cymene	0.48	-
10	6.416	Cymol	-	0.06
11	6.4223	o-Cymene	0.14	-
12	6.4643	Limonene	3.84	1.35
13	6.4672	Terpinolene	1.07	0.05
14	6.5231	Eucalyptol	-	1.02
15	6.6304	2-Pentyl acetate	0.44	-
16	7.2226	Undecanol	0.09	-
17	7.401	α-Terpinene	0.3	0.25
18	7.4888	3-tert-Butylphenol	0.8	-
19	8.1825	α-Dichlorohydrin	0.25	-
20	8.4235	1-Methylindene	-	0.14
21	8.7599	4-Terpinenol	0.13	0.07
22	8.8723	Carvacrol	0.04	29.6
23	8.9502	α-Terpineol	0.51	1.05
24	9.0506	Benzyl Alcohol	0.08	-
25	9.4819	3-Methyl-2-cyclopenten-1-one	0.02	-
26	10.2977	Borneol	0.56	1.6
27	10.5184	1-Hexadecene	0.12	0.26
28	10.6349	1-Tridecene	0.21	-
29	10.8868	1,3-Diisopropylbenzene	-	0.15
30	11.1312	3-Carene	11.5	-
31	11.4449	Bromomethane	0.04	-
32	12.0372	o-Xylol	-	0.9

33	12.1627	Longifolene	2.3	54.4
34	12.3899	3-Isopropylphenol	-	0.6
35	12.8008	Pentadecane	0.1	0.16
36	13.2035	2,4-Di-tert-butylphenol	0.14	-
37	13.6615	1-Tetradecanol	0.1	-
38	13.6952	2,6-Dimethylquinoline	0.43	-
39	13.6998	Quinoline	-	0.1
40	13.8372	Nerolidol	23.09	2.5
41	14.3252	4-tert-Butylphenol	5.96	-
42	14.4201	N,N-Diethyl-p-phenylenediamine	0.3	-
43	14.5126	Pyrethrin I	2.82	-
44	14.5211	Menthol	3.75	-
45	14.6216	4-tert-Butylbenzoic Acid	-	1.17
46	15.9036	α-Tridecene	-	0.12
47	16.1073	Hexadecanol		0.2
48	16.9177	2-Nonanone	0.09	-
49	17.2671	1-Hexadecanol	0.39	-
50	17.7234	Methyl hexadecanoate	-	0.28
51	18.1397	Dibutyl phthalate	0.17	0.03
52	18.3836	Ethyl hexadecanoate	-	0.15
53	19.4038	Methyl oleate	0.17	0.08
54	19.4758	γ-Decalactone	2.62	-
55	20.2006	Tetracosane	1.45	-
56	21.1167	Tridecane	0.08	
57	23.1806	Bis(2-ethylhexyl) phthalate	-	0.7
58	24.2669	Hexadecane	0.58	-
		Total oil (%)	**99.78**	**99.65**



A **B**

Fig.1-GC-MS chromatogram of (A) Leaves and (B) Bark oil of *Cryptocarya stocksii*

4.2. Anti-lipid peroxidation activity

Lipid peroxidation is widely recognized as primary toxicological event, which caused by the generation of free radicals from a variety of sources including organic hydro peroxides, redox cycling compounds and iron-containing compounds. In present work the TBARS assay was used to measure the degree of lipid peroxidation. TBA reacts specifically with malondialdehyde (MDA), a byproduct of lipid peroxidation to give a red chromogen, which can be read by spectrophotometrically [50]. The essential oil from leaves and bark of *C.stocksii* was capable to prevent the formation of MDA at dose dependent manner (Fig. 2) and IC_{50} found to be leaves 50.94±1.70 µl/ml and bark 33.49±1.52 µl/ml and standard α-lipoic acid were found to be 9.45±2.238 µg/ml (Tab.3). These result suggest that, the oil might prevent reactive radical species from damaging biomolecules such as DNA, proteins, lipoprotein, amino acids, sugar, and PUFA in biological and food systems.

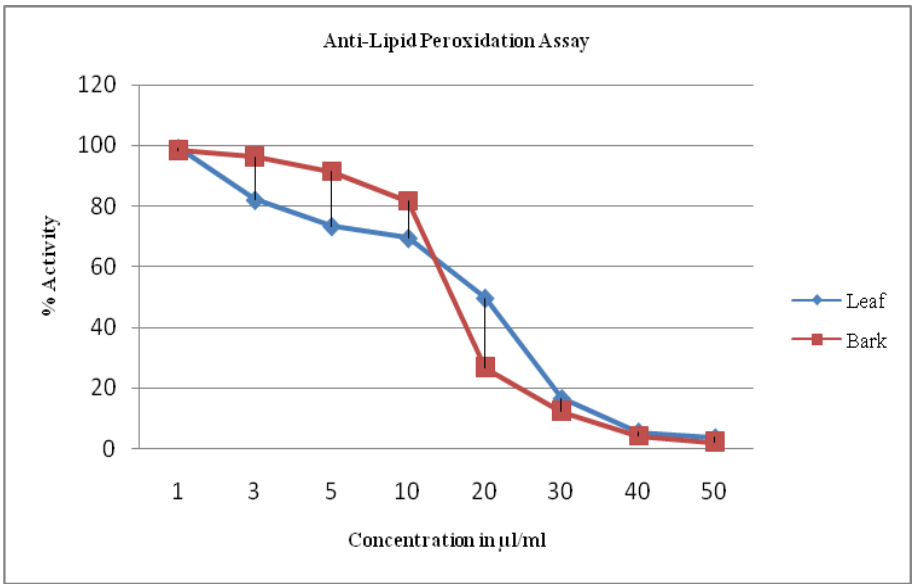


Fig-2. Anti-lipid peroxidation activity of leaves and bark essential oil (µl/ml), isolated from *C.stocksii.*

4.3. DPPH free radical scavenging activity

The result showed a dose-dependent inhibition of DPPH radical. DPPH free radical is stable nitrogen centered free radical commonly used for testing radical scavenging activity of the plant extracts. The violet colour of the DPPH radical reduced to yellow colored diphenylpicrylhydrazine radical after accepting an electron from the antioxidant compound, which is measured spectrometrically. Substances which are able to perform this reaction will be considered as antioxidants and therefore radical scavengers [51].

DPPH radical scavenging activity of essential oil from *C.stocksii* was denoted in Fig. 3 over the range of 1-50 µl/mL concentration and the IC_{50} value was found to be 33.88±1.53 µl/ml and 26.73±1.42 µl/ml leaves and bark respectively Correspondingly, IC_{50} value for ascorbic acid, used as standard, was 3.29 µg/ml ±2.91 (Table.3).

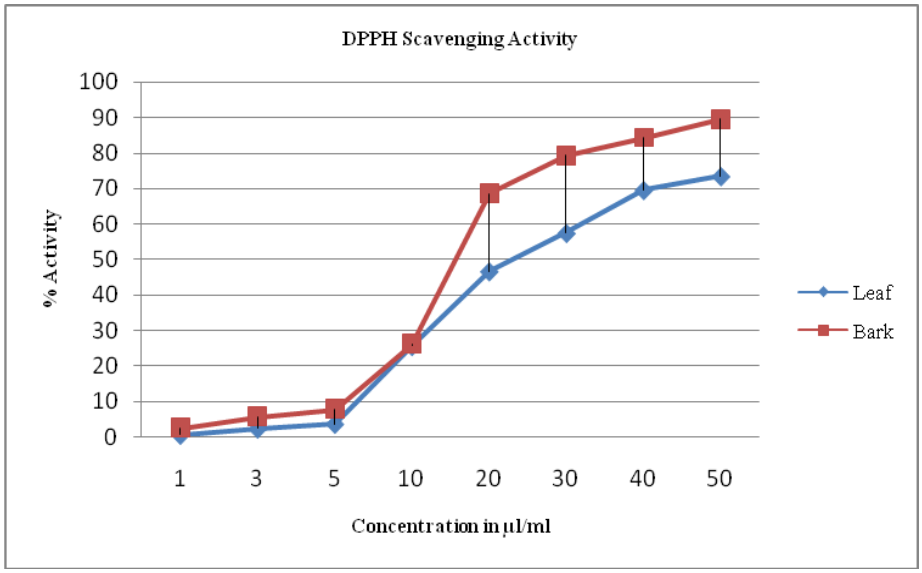


Fig-3.DPPH radical scavenging activity of leaves and bark essential oil (µl/ml), isolated from *C.stocksii.*

4.4. Hydrogen Peroxide assay

Several research reveals that superoxide anions

damages the biomolecules directly or indirectly by forming H2O2, ¯OH, singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation [52, 53]. H_2O_2 is a non-radical reactive oxygen species and the most stable intermediate in the four-electron reduction of O_2 to water [54]. Since H_2O_2 is uncharged, it easily passes through cell membranes by diffusion, and when inside the cells it can react with transition metals generating hydroxyl radicals (HO.). At high concentrations, these radicals induce peroxidation of lipids and proteins, affecting cell integrity [55]. Thus, exploring essential oil behavior in the presence of H_2O_2 remains of high interest. However, the required concentrations (Fig.4) for the inactivation of 50% of the H_2O_2 are: Leaves 48.08±1.68 µl/ml and bark 58.06±1.76 µl/ml for essential oil. Whereas, IC_{50} value for quercetin, used as standard, was 19.59 µg/ml ±3.23 (Tab.3).

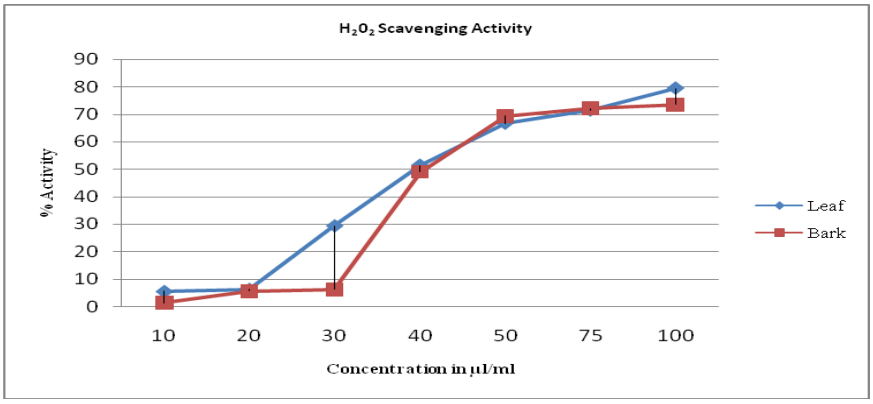


Fig-4. *Hydrogen Peroxide scavenging* activity of leaves and bark essential oil (µl/ml), isolated from *C.stocksii.*

4.5. Reducing Power assay

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant action [56]. The reducing ability of a phytochemical generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom [57]. Reducing power of the essential oil increases with the increase in concentration and showed good reducing power ability in a dose dependent manner (Fig.5) with IC_{50} of leaves 74.63±1.87µl/ml and bark 39.21±1.59 µl/ml. IC_{50} value for BHA, used as standard, was 12.76 µg/ml ± 1.45 (Tab.3). The antioxidant principles present in oil cause the reduction of Fe3+/ferricyanide complex to the ferrous form and thus proved essential oil isolated from *C. stocksii* has promising radical scavenging ability and potent reducing power.

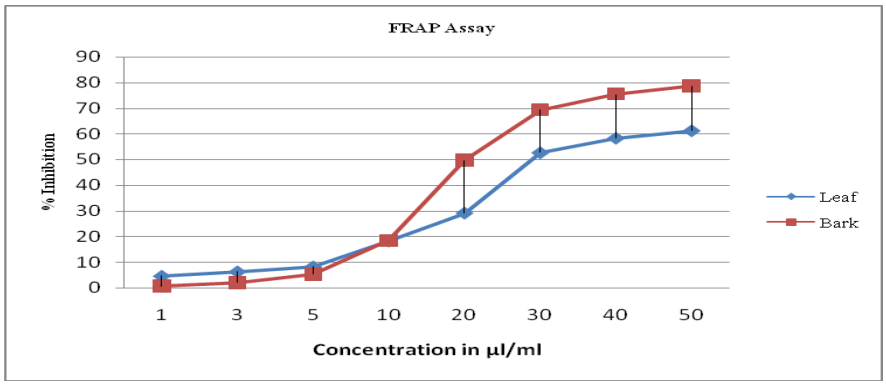


Fig-5.Reducing power activity of leaves and bark essential oil (µl/ml), isolated from *C.stocksii.*

Table-3.Antioxidant capacity of essential oils of *C.stocksii* .Values are mean ± SE.

	IC_{50} value (µl/ml)		IC_{50} value (µg/ml)
Activity	Leaves	Bark	Standard Antioxidants
FRAP Assay	74.63±1.87	39.21±1.59	12.76±1.45
ALP Assay	50.94±1.70	33.49±1.52	9.45±2.238
DPPH Scavenging Activity	33.88±1.53	26.73±1.42	3.29±291
H_20_2 Scavenging Activity	48.08±1.68	58.06±1.76	19.59±3.23

5. CONCLUSION:

This study has been concerned with determining the chemical composition and antioxidant activity characteristics of essential oils isolated from *C. stocksii* leaves and bark collected in the Western Ghats region of Karnataka. The study concludes that the essential oil of *C stocksii* is a very good source of terpenes. The oil exhibited dose dependent manner of antioxidant activity thereby implying its potential in providing protection against oxidative diseases and its use as a natural antioxidant in the food and confectionery industries and future clinical studies are needed in order to ascertain their therapeutical value.

6. ACKNOWLEDGEMENT

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