PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF VARIOUS MEDICINAL PLANT EXTRACTS

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Article History: Received: 28.10.2021 **Revised:** 13.12.2021 **Accepted:** 20.01.2022

Abstract

Kigelia pinnata, *Cichorium intybus* and *Cedrous deodara* are traditionally used medicinal plants against several diseases. This study aims to undergo qualitative, quantitative and antioxidant properties of several extracts from these plants using in-vitro assay. Qualitative study of extracts thin layer chromatography (TLC) was carried out. Quantitative analysis to determine total content of phenolic, flavonoid, iridoid and terpenoid using specific chemical reagents. DPPH (Free radical diphenylpicrylhydrazyl), Phosphomolybdate (Total antioxidant capacity) and reducing power assay were used to measured antioxidant potential to each extracts. TLC revealed presence of phenylpropanoids, naphthaquinones, flavonoids, phenolics, terpenoids, volatile oils, organic acids in different extracts. High phenolic content was found in methanolic extract of *C. intybus* and *K. pinnata* fruit root (11.97 ± 0.37 and 11.11 ± 0.37 mg/g). Flavonoids were higher in both ethanolic and methanolic extracts of *C. intybus* root (6.12 ± 0.32 and 5.80 ± 0.96 mg/g). Terpenoid content was higher in ethanolic extract of *C. deodara* wood oil (5.714 ± 0.71 mg/g). Iridoids were found only in methanolic extracts of *K. pinnata*. With the exception of methanolic extract, all extracts had high free radical scavenging properties, but they were all noticeably less effective than ascorbic acid. Reducing power activity was significantly lesser in all extracts as compared to Quercetin. The extracts are rich in various important phytochemicals and seem to have good antioxidant potential but need to be assessed using biological assays.

Keywords: antioxidant activity, DPPH, Phytoconstituents, Phosphomolybdate, Reducing power, Thin layer chromatography

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DOI: 10.53555/ecb/2022.11.01.30

1. INTRODUCTION

Oxidative stress is a condition leads to cell and tissue damage by generating excessive reactive oxygen species.[1] In Mitochondria, ROS generates during its metabolic process cause oxidation of mitochondrial proteins, lipids and DNA accompanies to crippling diseases including obesity, diabetes, hypertension, cancer, cardiovascular, inflammatory and neurodegenerative disorders.[1,2] Antioxidants play a crucial role for prevention of ROS associated disease via restraining or neutralising the resulting free radical.[1] Recent studies indicate antioxidants obtained from plants have ability to scavenge free radicals may be of great therapeutic value. Most of the medicinal plants consist phytoconstituents i.e., flavonoids, phenolics, terpenoids, alkaloids, volatile oil etc. These phytoconstituents scavenge free radicals and may have antioxidant property. Many synthetic antioxidants are also available but they have harmful and/or mutagenic adverse effects used for longer period of time.[3] So, the plant-based medications widely used to reduce side effects other than the synthetic medication.

Kigelia pinnata an indigenous medicinal plant traditionally known as "Balamkheera" residing to Bignoniaceae family. Tree is native to African continent widely begin in southern, central and western areas especially drove to various south-east Asian nations including India, Pakistan, China, Philippines and Iraq.[4] In India it is widely distributed in Purna River - Navsari region of south Gujarat. The mature fruits are amphisarca hanging on the tree in groups of 2-3 on very large rope-like peduncles contains phenylpropanoids, iridoids, phenolic, flavonoids, naphthaquinones, terpenoids etc. This plant having antibacterial, antifungal, diuretic, anticancer, anticonvulsant, antidiabetic, antioxidant, anti-inflammatory and analgesic activity.[5]

Chicory, also known as *Cichorium intybus L.*, perennial herb pertains to family Asteraceae.[6] Taproot of chicory widely grown in Chile, India, South Africa and northwest Europe. Ancient Egyptians cultivated chicory as a vegetable crop, a coffee replacement, a medicinal herb and occasionally as animal feed. The chicory root consist of 98% inulin (polysaccharide) and 2% of other compounds including anthocyanidins, flavonoids, polyphenols, sesquiterpenoids, organic acid, sesquiterpene lactones, triterpenoids and volatile oil. Several health-promoting properties of chicory roots include antioxidant, anti-obesity, anti-hepatotoxic, anti-carcinogenic, anti-inflammatory, antiviral, antibacterial and anthelmintic action.[6,7] Due to its abundant supply of inulin, a water-soluble fibre that cannot be digested and used as fat substitute in various processed and functional foods. Some regions of U.S. chicory root coffee widely popular.[8] The USFDA provides nutritional values of *C. intybus* contains calories: 32, 0.1g fat, 22.5mg sodium, 7.9g carbohydrate, 0.7g fibre, 3.9g sugars, 0.6g protein, 130mg potassium, 18.4mg calcium, 27.4mg phosphorus and 10.4µg folate.[8]

Another traditional folk plant, *Cedrus deodara* belongs to family pinaceae commonly known as 'Devdaru'. *C. deodara* found in himachal Pradesh regions of Kangra (Bada & Chota Bhangal), Mandi, Kinnaur, Sirmour, Shimla and Chamba.[9] Traditionally, the qualities of heartwood include diuretic, expectorant, diaphoretic, carminative and antioxidant effects. A wood decoction used to treat fevers, pulmonary, urinary and gastrointestinal disorder, rheumatism, piles, kidney stones, sleeplessness, diabetes, etc.[10] It has been employed as a remedy for snake bites. By distilling the wood, obtained essential oil has used in the treatment of phthisis, bronchitis, rheumatism, blennorrhagia and skin eruptions.[11] A wood-derived resin is applied externally to treat skin conditions, joint injuries and bruising.[9,11] The leaves are employed in Ayurvedic medicine to cure tuberculosis. The seed oil extract has diaphoretic properties.[11]

The goal of this research was to conduct qualitative estimation of preliminary screening for phytoconstituent rich different plant extracts by TLC, quantitative analysis of phytoconstituent-rich extract was conducted to determine total amount of phenolic, flavonoid, iridoid and terpenoid as well as the in vitro antioxidant properties.(by DPPH, TAC and RP). As far as we are aware, there has not been any specific research on this phytoconstituent-rich extract.

2. MATERIAL AND METHODS

2.1 Sample collection and its authentication*:*

K. pinnata fruit was assemble riverine area of south Gujarat region-Navsari. The authentication of *K. pinnata* fruit, its botanical identification and voucher specimen (No.12/BOT/22) was confirmed by Dr. Alpna Shukla, Department of botany, M. G. Science Institute, Ahmedabad, Gujarat. *Cichorium intybus* root power procured with its CoA No.0287/21-22 from pioneer chicory hub, Anand, Gujarat. *Cedrous deodara* wood collected from Hakim chichi, Surat authentication and its specimen no.GU/BOT/P/C/01 provide Prof. Hitesh Solanki who works in Department of Botany, Gujarat University, Ahmedabad, Gujarat.

(a) (b) (c)

Figure 1. Representation of collection of plant part: (a) Kigelia pinnata – Fruit, (b) Cichorium intybus – Root and (3) Cedrous deodara – wood

2.2 Preparation of various phytoconstituents rich plant extracts:

2.2.1. Extraction of *K. pinnata* fruit:

The fruits were cleaned with distilled water, peeled, sliced into little pieces and dried 16 days at 40^0C. The dried fruits were milled by using hammer mill after powder were passing through a sieve no. 40 and 60 for collection of fine powder. Store in dark, closed containers at room temperature until used.

(a) Extraction of *K. pinnata* fruit by using Dichloromethane (KPDCME): 50gm of *K. pinnata* fruit powder extracted using Soxhlet extraction with 170ml of Dichloromethane as a solvent for 8 hrs. The extract was concentrated at room temperature for 20 min.[12]

(b) Extraction of *K. pinnata* fruit by using methanol (KPME): The conical flask was filled with 20g of the fruit powder from *K. pinnata*, and 600 ml of methanol was then added. Supernatants were decanted into centrifuge tubes after the closed conical flasks were agitated for 30 minutes in the rotary shaker. The tubes were put into pre-weighed, clearly labelled glass vials after being centrifuged at 4.4 g for 20 min. conical flask was left open in fume cupboard to desiccating its solvent.[13] weighed vials again after drying, to calculate the amount of processed crude extracts.

2.2.2. Extraction of *Cichorium intybus* root:

(a) Extraction of *C. intybus* root by using methanol (CIME): 10gm of chicory root powdered were weighed and Soxhlet apparatus fitted with a 500ml round bottomed flask consist 250ml of methanol. Extraction was performed at boiling temperature of methanol (64.7^0C) for 6hr.[14] By employing a rotary evaporator, the solvent was evaporated after the filtrates were gathered, pooled, and collected.

(b) Extraction of *C. intybus* root by using ethanol (CIEE): Dry chicory root powder (25gm) was extracted using 250ml of 95% ethanol (24^0C) at RT for 24hr.[15] Solvents were evaporate under vacuum dried the extract and stored at 4^0C.

(c) Extraction of *C. intybus* root by using ethyl acetate (CIEAE): 25gm of *C. intybus* root powder extract with 250ml absolute alcohol at 24^0C for 24hr, defatted with n-heptane and then extract 250ml of ethyl acetate.[15] Solvents were eliminate using vacuum. The dried, concentrated dark syrup was collected, and kept at 4^0C.

(d) Extraction of *C. intybus* root by using hexane (CIHE): The dried 25gm root powder soaked separately in 100 ml of hexane containing conical flask, placed in rotary shaker for 3 days.[16] Extracts were purified via Whatman filter paper no. 1, stored at room temperature before being concentrated using a rotary evaporator under vacuum.

2.2.3. Extraction of *C. deodara* wood (CDO):

C. deodara 100gm wood powder placed Clevenger-type apparatus under 200ml of ethanol and distilled 6hr.[17] The oil had been placed in sealed containers once it dried on anhydrous sodium sulphate and kept at room temperature. The % yield has been calculated by employing equation given below:

$$\%\ \boldsymbol{yield} = \frac{\boldsymbol{gm\ of\ extract}}{\boldsymbol{gm\ of\ sample\ taken}} \times \mathbf{100} \quad (1)$$

2.3 Chemicals and Drugs:

DPPH, quercetin, Gallic acid, rutin, linalool, catalpol, ascorbic acid, sodium carbonate, Trichloroacetic acid, potassium ferricyanide, sodium phosphate, ferric chloride, ammonium molybdate, potassium hydroxide, Foline- Ciocalteu reagent, potassium acetate, aluminium chloride, copper sulphate, Anisaldehyde, vanillin, sodium

sulphate, ethyl acetate, acetone were procured from sigma Aldrich. Trifluoro acetic acid, glacial acetic acid, dichloromethane, n-butanol, petroleum ether, n-heptane, hexane, toluene, formic acid, 2-butanol, cyclohexane, dinitrobenzene, bromocresol green, 4-hydroxybenzaldehyde, butyl acetate, acetic anhydride, ethanol, methanol, chloroform, isopropanol were bought from Merck. For this work, only analytical-grade chemicals and reagents were employed.

2.4 Qualitative estimation of phytoconstituent rich extract through Thin-layer chromatography (TLC): 1mg plant-based extracts was dissolved in 1ml of its extracted solvent to make the stock solutions. For TLC study, stationary phase consist aluminium-backed silica coating and a different solvent system as the mobile phase. A small number of samples were discovered 0.5 cm from the TLC plate's bottom and laid down in the chamber, which was then allowed to fill for 15 minutes with solvent vapour. Five minutes were allotted for the development process. The plate was then taken out of the room and allowed to dry in the air. The plates were spray with specific reagent to observe the spot under UV 254 and 366 nm. Retention factor (Rf) was determined after coloured regions had been marked by applying below equation:

$$\textbf{Rf value} = \frac{\textbf{distance traveled by sample}}{\textbf{distance traveled by solvent front}} \quad (2)$$

2.5 Quantitative estimation for phytoconstituent rich extracts:

1) Total Phenolic Content (TPC):

Each plant extracts has been assessed to measure overall presence of phenolic molecules using a modified Folin-Ciocalteu colorimetric method. Each extract (0.2ml) has been incorporated with Folin-Ciocalteu reagent (0.6ml), 0.2 ml of the sodium carbonate solution (20% w/w), and dilute it to 10ml distilled water. Allow to incubate in the dark for 90min. Intensity of absorbance was observed 760nm through UV-VIS spectrophotometer.[18] Same technique used to derive curve for calibration of gallic acid (reference) in a concentration range 0.2-1 ng/ml. The outcomes were represented as mg Gallic acid equivalent per gm of dry extract (mg GAE/gm extract). Assay was performed in triplicate. (R^2 = 0.9993)

2) Total flavonoid content (TFC):

Entire existing flavonoid content in all plant extracts assessed using a few modification of previous AlCl3 colorimetric technique. Curve calibration ranging from 0.2–1 ng/ml chosen using rutin (reference). Each extract (0.2 mL) was combined with 1M potassium acetate (0.1 ml), 0.1ml - 10% AlCl3, 1.5 ml 95% ethanol, diluted with the appropriate solvent up to 10 ml in each case. Allow it to incubate 30 min. at RT.[18] Absorbance has been measured 451 nm. TFC was calculated rutin equivalents per gramme of extract (mg RE/gm extract). Assay was performed in triplicate manner. (R^2 = 0.997)

3) Total iridoid content (TIC):

The presence of iridoids was determined by combining 2 ml of extract with 10 ml Trim-Hill reagent (acetic acid, Copper sulphate- 0.2%, conc. hydrochloric acid - 10:1:0.5). An absorbance measurement was made at 609 nm. Only methanolic extract of K.pinnata fruit shows blue colour indicate presence of iridoids.[18] Testing was done in triplicate. TIC of plant extracts were determine catalpol equivalents per gm of dry extract (mg CE/gm extract) as standard with range of 0.2–1 ng/ml for calibration curve. (R^2 = 0.9905)

4) Total terpenoid content (TTC):

0.2 ml extract dissolved 2 ml of its solvent for all three plant extracts to determine the terpenoid content. The mixture was completely vortexed after which it was allowed to stand for 3 minutes. Then, 200 µl of conc.H2SO4 added, allow to stand at RT for 2 hr. During incubation, a reddish-brown precipitate was visible in the mixture. In order to avoid disrupting the precipitate, the supernatant was carefully decanted. 3ml of 100% methanol was then subsequently added, mixture was vortexed until the precipitation had completely dissolved.[19] UV-visible spectrophotometer had been employed to assess absorbance at 538nm. TTC of plant derived extracts was calculated as mg linalool equivalent per gm of extract (mg LE/ gm). (R^2 = 0.9963)

2.6 In vitro studies:

Different plant extracts' antioxidant capacity was assessed using DPPH, Phosphomolybdenum (Total antioxidant capacity) and Reducing power assay compared with standard compound i.e., ascorbic acid and quercetin.

1) 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: [20,21]

Principle: Due to the free radical delocalization in aromatic rings, the DPPH radical is renowned for its extraordinary stability. Its colour is an extreme deep purple. In tests, radical is neutralised by absorbing an atom of hydrogen/electron via antioxidant plant extracts (or reducing substance). (DPPH or DPPH-H). A rich purple colour results from the DPPH radical's unpaired electrons significantly absorb at 517 nm.[20] Whenever an odd electron interacts with

another electron, the original coloured transforms to a pale yellow.

Procedure: 2 ml of DPPH (0.1 mM) added in 2ml of both standard as well as sample. After vigorous stirring the mixture for two minutes, incubate 30 min. at RT kept in dark. Standard (ascorbic acid) and sample concentration range were prepared. (0.2-1 ng/ml). Decolorization of DPPH has been evaluated to record absorbance at 517nm in contrast to methanol (blank) using UV spectrophotometer.[22]

2) Phosphomolybdate assay (Total antioxidant capacity):[21,22]

Principle: The production of Phosphomolybdenum compound, a spectroscopic approach used to estimate overall antioxidant ability. The method employed depends on analyte of sample reduced transformation of Mo (VI) to Mo (V), followed by developing green phosphate Mo (V) complex.

Procedure: 1ml of standard (ascorbic acid) & sample solution were incorporated in 1ml reagent consist (0.6 M sulfuric acid, 28 mM sodium orthophosphate and 4 mM ammonium molybdate), allow to incubate 90min at 95°C and tubes were cooled. Tubes were kept in ember colour container covered with silver foil. Record absorbance at 765 nm against a blank. (Distilled water).

3) Reducing power assay:[21,22]

Principle: The reduction of (Fe3+) is frequently utilized as a biomarker of donating electrons, a key predictor ability to reduce phenol. Reduced herbal extracts interact with potassium ferricyanide (Fe^{3+}) to produce potassium ferrocyanide (Fe^{2+}), which in turn reacts with ferric chloride in order to produce ferric-ferrous complex exhibiting an absorbance threshold at 700 nm.[22]

Procedure: sample and standard (quercetin) solutions were dissolved in its appropriate 1ml solvent. Add 1ml of phosphate buffer (0.2M, pH 6.6), 1ml potassium ferricyanide (1%) solution and incubated at 50^0C for 20 minutes followed by 1ml Trichloroacetic acid (10%) was introduced for terminating the reactions. Tubes were allowed to cool for 5 min. centrifuge 3000 rpm at 10 min. 2 mL of supernatant was discarded in addition to reconstitute using 2ml distilled water along with 0.4 ml of ferric chloride (0.1%). Absorption for the solution being examined at λmax 700nm. Increased rate of absorbance indicates sample have more efficient reduction capacity.

Calculations: Antioxidant activity (using DPPH, Phosphomolybdenum and reducing power) was determined using formula shown below:

$$\% \textbf{ Reactive scavenging activity} = \frac{(\mathbf{A0}-\mathbf{A1})}{\mathbf{A0}} \times \mathbf{100} \quad (3)$$

Whereas, A0 - absorbance of control blank and
A1 - absorbance of test/standard.

3. RESULT AND DISCUSSION

3.1 Sample extraction:

Depending up on the polarity of different solvents plant extraction was carried out by using soxhlet, simple maceration and Clevenger type methods. The amount of plant material used for extraction and obtained yield enumerate in Table-1.

Table 1. Obtained % yield of plant extraction using various solvents

Sr no.	Phytoconstituents rich extract	Amount of plant materials used (gm)	% Yield
1	Methanolic extract of *K. pinnata* fruit (KPME)	20	10.97
2	Dichloromethane extract of *K.pinnata* fruit (KPDCME)	50	36.5
3	Methanolic extract of *C. intybus* root (CIME)	25	21.28
4	Ethanolic extract of *C. intybus* root (CIEE)	25	23.56
5	Ethyl acetate extract of *C. intybus* root (CIEAE)	25	19.8
6	Hexane extract of *C. intybus* root (CIHE)	25	18.88
7	Ethanolic extract of *C.deodara* wood (CDO)	100	18.5

3.2 Assessment of Phytochemicals:

The following phytoconstituents were detected using TLC utilising various solvent systems for plant extracts: (i) Methanolic extract of *K. pinnata* fruit (KPME) revealed presence of iridoids, phenylpropanoids, flavonoids, phenolics. (ii) Dichloromethane extract of *K.pinnata* fruit (KPDCME) presence naphthaquinones, (iii) Root of *C. intybus* methanolic extract (CIME) are rich anthocyanidins, flavonoids and polyphenols, (iv) *C. intybus* root ethanolic extract (CIEE) shows sesquiterpenoids and organic acid, (v) Ethyl acetate extract of *C. intybus* root (CIEAE) have rich fraction of sesquiterpene lactones, (vi) Hexane extract of *C.*

intybus root (CIHE) shows presence of volatile oil and triterpenoids, (vii) Ethanolic extract of *C. deodara* wood (CDO)consist rich fractions of sesquiterpenoids, triterpenoids, flavonoids and lignans. (Table 2)

Table 2. Screening of phytoconstituents rich extracts by thin layer chromatography (TLC).

Sr no.	Phytoconstituent rich plant extracts	Mobile phase	Detection	Observed under vis.	Observed under UV 365nm	Rf value	Presence of phytoconstituents
1	Methanolic extract of K. pinnata fruit (KPME)	Dichloro methane: methanol (8:2)	Anisaldehyde-sulphuric acid reagent	brown color	Blue fluorescence	0.43	Phenylpropanoids[23]
		water: ethanol: n-Butanol (0.5:1.5:8)	Vanillin-Sulphuric acid reagent (VS)	Extract show Violet blue colour, Catalpol shows violet blue colour	-	0.72, Standard (catalpol) - 0.66	iridoids[24]
		ethyl acetate: petroleum ether (3:7)	1% ethanolic aluminium chloride reagent	yellow colour band	Yellow fluorescence	0.65, Standard (Rutin) - 0.17	flavonoids[25]
		Ethyl acetate : methanol (9:1)	Foline Ciocalteu reagent and sodium carbonate solution.	blue colour	blue fluorescence	0.74, Standard (Gallic acid - 0.86)	phenolic[26]
2	Dichloromethane extract of *K.pinnata* fruit (KPDCME)	CHCl3: isopropanol (19:1)	10% methanolic KOH reagent	red to red-brown color	red fluorescence	0.3	naphthaquinones[12]
3	Methanolic extract of *C. intybus* root (CIME)	Toluene: ethyl acetate: glacial acetic acid (5:4:1)	1% ethanolic aluminium chloride reagent	-	Yellow fluorescence	0.91, Standard (Rutin)- 0.88	flavonoids[27]
		Toluene: Acetone: formic acid (4.5:4.5:1)	Foline Ciocalteu reagent and sodium carbonate solution.	blue colour	blue fluorescence	0.83**,** Standard (Gallic acid)- 0.73	phenolic[27]
		ethyl acetate: 2-butanol: 0.1mM trifluoroacetic acid in methanol: water (3:3:2.5:1.5)	Anisaldehyde-sulphuric acid reagent	deep purple colour	-	0.76	anthocyanins[28]
4	Ethanolic extract of *C. intybus* root (CIEE)	Cyclohexane: ethyl acetate:	Zimmermann reagent	Violet grey colour	-	0.85	Sesquiterpenoids[29]

		acetic acid (6:3:1)					
5	Ethyl acetate extract of *C. intybus* root (CIEAE)	butyl acetate: ethyl acetate: 1-butanol: acetic acid (4.5:1.5:2.5:1.5)	Zimmermann reagent	Violet grey	Blue zone	0.52	sesquiterpene lactones[30]
6	Hexane extract of *C. intybus* root (CIHE)	Hexane: ethyl acetate (8:2)	Komarowsky reagent	-	Blue fluorescence	0.975	Volatile oil[31]
		Ethyl acetate: methanol (6:4)	Liebermann-Burchard reagent	blue violet colour	-	0.775, Standard (linalool) -0.46	Triterpenoids[32]
7	Ethanolic extract of *C.deodara* wood (CDO)	n-hexane: ethyl acetate (9:1)	Liebermann-burchard reagent	reddish brown colour	reddish brown fluorescence	standard (linalool) -0.93, 0.46	Terpenoids[33]
		toluene: ethyl acetate: acetic acid (6:3.5:0.5)	5% solution of sulphuric acid in ethanol, followed by 2 min heating at 150^0 c	violet colour	-	0.58	Lignans[34]
		cyclohexane: ethyl acetate: acetic acid (6:3:1)	Zimmermann reagent	violet grey colour	-	0.83	Sesquiterpenes[29]
		Dichloro methane: methanol: water (6:3.8:0.2)	1% ethanolic aluminium chloride reagent	yellow colour band	yellow fluorescence	standard (rutin)-0.86, 0.95	flavonoids[35]

3.3 Total phenolic, flavonoid, iridoid and terpenoid content:

Phenolic compounds (PCs) function as antioxidants by interacting with various types of free radicals which able to catalyse the oxidation reaction.[36] Plant polyphenols are a substantial category of chemicals that serve as major antioxidants or scavengers of free radicals. A substituted hydroxyl group surrounds the aromatic benzene ring in polyphenol substances include their functional offspring. Free radicals can be absorbed by them, they could chelate metal ions, stimulate production of reactive oxygen species and encourages lipid oxidation products.[36,37] Our total phenolic content result revealed that methanolic extract of *C. intybus* root have higher amount 11.97 mg GAE/g of extract, low amount found methanolic extract of *K. pinnata* fruit 11.11 mg GAE/g of extract and least amount in ethanolic extract of *C. intybus* 1.82 mg GAE/g of extract. Mechanism underlying phenolic constituents as antioxidant based on the transfer of a hydrogen atom, single electron and proton loss occur after sequential transfer of an electron or chelation of transition metals.[36] The higher amount of phenolic constituent's presence in methanolic extract of *C.intybus* demonstrates excellent antioxidant activity may possess more capacity to neutralise free radicals.

Flavonoids significantly support the body's ability to fight against disease. Based on locations of the hydroxyl group, their molecular topologies, and other features of their chemical structures, flavonoids can operate as powerful antioxidants. As its glycoside, they are widely distributed throughout plants.[38] Ethanolic extract of *C.intybus* root shows higher level of flavonoids 6.12 mg rutin/gm of extract as compared to methanolic extract of *C.intybus* 5.80 mg rutin/gm extract and methanol extract from *K.pinnata* fruit found 2.36 mg RE/gm extract. Least amount of flavonoid shown in

C.deodara oil 1.23 mg rutin/g of extract, while the other extract not possess the flavonoid content. Generally, flavonoids are polyphenol constituent the oxidation of its phenolic moieties indirectly involved expression of genes increase cell endogenous antioxidant capacity by interaction with the generation ROS.[38]

Total terpenoid content of *C.deodara* wood oil was found higher amount 5.714 mg LE/gm extract as compared to hexane extract of *C. intybus* 2.063 mg LE/gm extract while the other plant extract not have terpenoid content. Terpene/steroidal substances have capacity to influence apoptosis and a number of different cell death mechanisms through manipulation of ROS.[39] Triterpenoids alter the function of mitochondrial membrane through cell death regulating mechanism of autophagy by intrinsic (caspase 3/9) and extrinsic pathway (caspase 8) inducing apoptosis and have a role as an antioxidant to snuff out ROS.[39]

Iridoids are a subclass of monoterpenoids acts as antioxidant by reducing oxidative stress. According to literature survey, iridoids scavenge ROS in Alzheimer's disease by targeting chelation of direct metals.[40] Only methanolic extract of *K.pinnata* fruit consist total iridoid content 13 mg catalpol/g of extract as compared to other plant extract. Hence, it may be concluded that polyphenol and flavonoid compounds may combine with other phytoconstituents of active plant extracts to confer therapeutic significance.

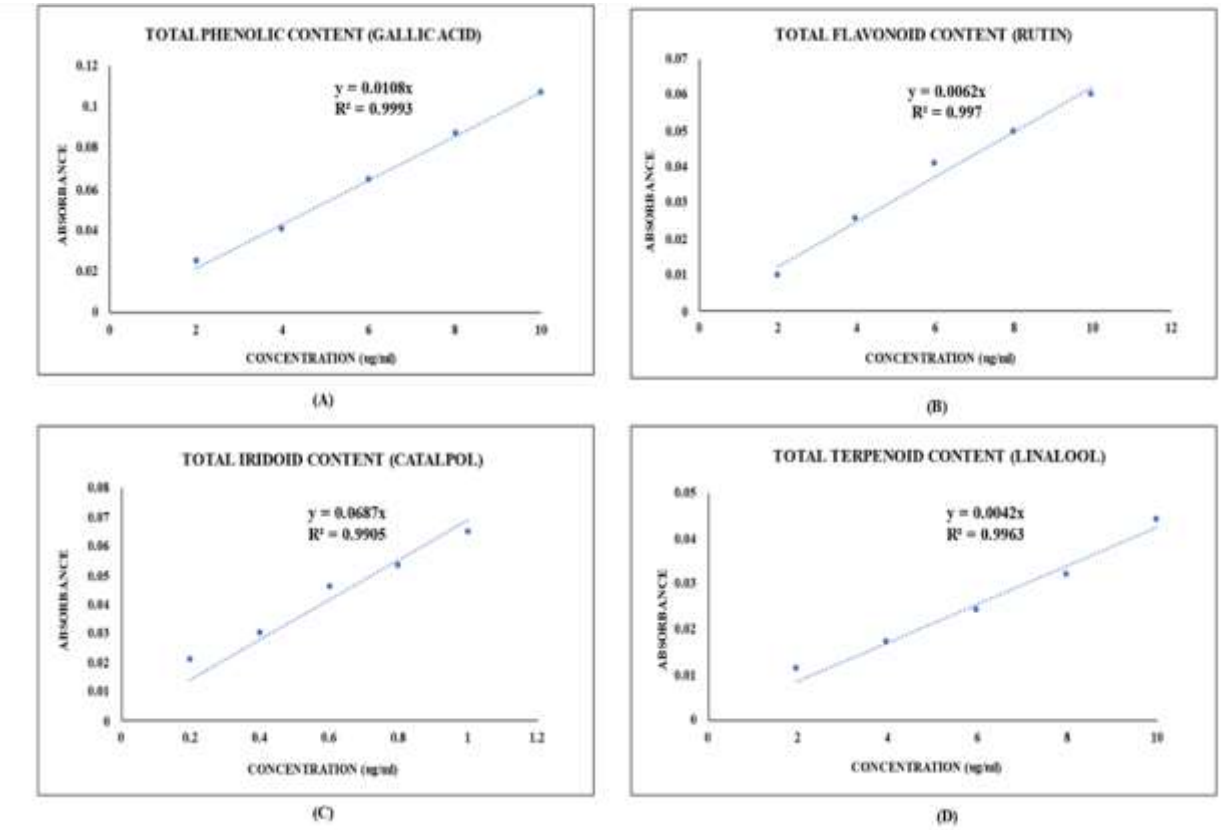

Figure 2. Standard curve for determine Total content of (A) Phenolic (Gallic acid), (B) flavonoid (Rutin), (C) Iridoid (catalpol) and (D) terpenoid (linalool)

Table 3. Quantitative estimation of various phytoconstituents rich extracts

Sr no.	Phytoconstituents rich extract	Phenolic (mg GAE/gm extract)	Flavonoid (mg RE/gm extract)	Iridoid (mg CE/gm extract)	Terpenoid (mg LE/gm extract)
1	KPME	11.11 ± 0.27	2.36 ± 0.56	13.0 ± 0.006	-
2	KPDCME	-	-	-	-
3	CIME	11.97 ± 0.37	5.80 ± 0.96	-	-
4	CIEE	1.82 ± 0.374	6.12 ± 0.32	-	-
5	CIEAE	-	-	-	-
6	CIHE	-	-	-	2.063 ± 2.08
7	CDO	-	1.23 ± 0.40	-	5.714 ± 0.71

3.4 Determination of IC50 value by Antioxidant assays:

Plants are known for their therapeutic benefits, having antioxidant by free radical scavenging qualities. With the help of three distinct assays - DPPH, Phosphomolybdenum (TAC) and reducing power found the antioxidant potential of phytoconstituent rich extract. Numerous variables, such as test equipment and content of plant extract affect ability of plant extract's function as antioxidants. Performing single experiment for assessment of antioxidant capabilities would not yield the accurate results. Owing to the diversity of antioxidant effectiveness mechanisms variety of antioxidant capacity tests must be carried out. This test allows us to assess the antioxidant potential of both hydrophilic and lipophilic compounds in a single sample. These three assays can produce repeatable results are highly straightforward, affordable and frequently used techniques for determining antioxidant activity.

The results obtained from antioxidant assays of DPPH, Phosphomolybdenum (TAC) and RP were derived using calibration graphs that had R^2 values of 0.9739, 0.8365 and 0.8198 respectively. From antioxidant assays, IC50 value of each extracts obtained which indicates lower the inhibitory concentration higher the antioxidant activity.

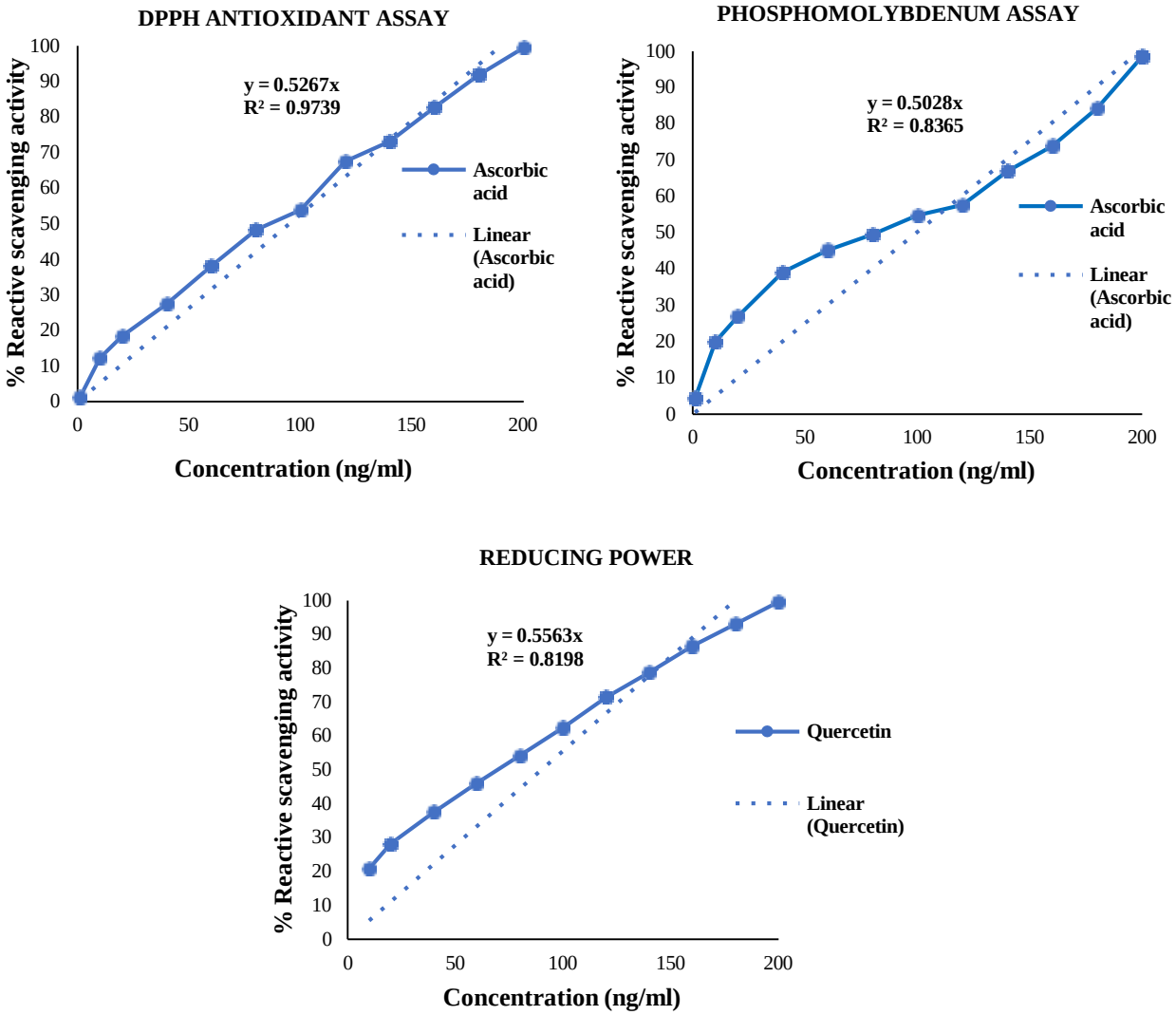

Figure 3. Standard curve of antioxidant assay: (A) DPPH assay (Ascorbic acid), (B) Phosphomolybdenum assay (Ascorbic acid) and (C) Reducing power assay (Quercetin).

Various crude active phytoconstituent rich extracts was determined its antioxidant effect by utilising DPPH method. Obtained outcome from the study shows standard ascorbic acid at concentration 1000 ng/ml has higher free radical scavenging activity (99.32%) relative to other extracts. Other extracts at same concentration scavenge % free radical observed CDO (73.14%), CIME (69.59%), CIEE

(65.20%), CIEAE (64.52%), CIHE (63.15%) and KPME (57.60%). The inhibitory concentration (IC50) of standard ascorbic acid possess 57.37 ng/ml while other extracts CDO (287.61 ng/ml), CIEAE (304.01ng/ml), CIEE (358.43 ng/ml), CIME (375.77 ng/ml), CIHE (595.03 ng/ml) and KPME (1529.36 ng/ml). The stronger antioxidant activity is shown by the lower IC50 value. From obtained results, DPPH assay indicates that ethanolic extract of *Cedrus deodara* wood (CDO) and ethyl acetate extract of *Cichorium intybus* root has more antioxidant power. This may be owing to the extracts high concentration of different phytoconstituents, which serve to neutralise the DPPH radical. Figure 4 represent DPPH assay of standard (ascorbic acid) and test extracts.

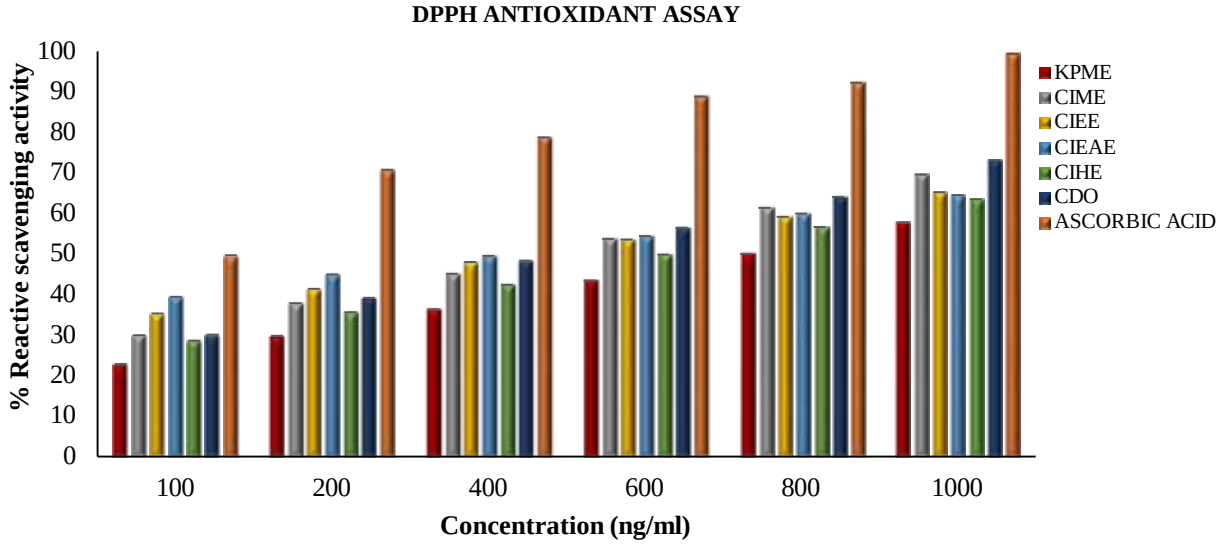

Figure 4. IC50 of Plant derived extracts scavenge DPPH radical. Values presented in terms of mean ± standard deviation at concentration ranges 100-1000 ng/ml. (n = 3)

Phosphomolybdenum, commonly known as total antioxidant capacity assay, suggested free radical scavenger action. Ethanolic extract of *Cedrous deodara* wood oil (98.84%) has more antioxidant effect than standard ascorbic acid (98.60%), while IC50 at concentration 1000 ng/ml permit 53.02 ng/ml and 56.22 ng/ml respectively. Free radical activity of other plant extracts exhibited methanolic extract of *kigelia pinnata* fruit (92.75%), ethyl acetate extract of *Cichorium intybus* root (97.94 %), ethanolic extract of *Cichorium intybus* root (86.086 %), hexane extract of *Cichorium intybus* root (85.217 %) and methanolic extract of *Cichorium intybus* root (78.84 %).The IC50 value found from these assay for CDO (53.02 ng/ml), ascorbic acid (56.22 ng/ml), KPME (61.21 ng/ml), CIEAE (64.44 ng/ml), CIEE (71 ng/ml), CIHE (75.81 ng/ml) and CIME (95.16 ng/ml). The presence of many excessive antioxidant rich phytoconstituents in the extracts it might be reduced Mo (VI) to Mo (V).[21] Here, extracts had higher IC50 indicates significantly weaker the total antioxidant capacity.

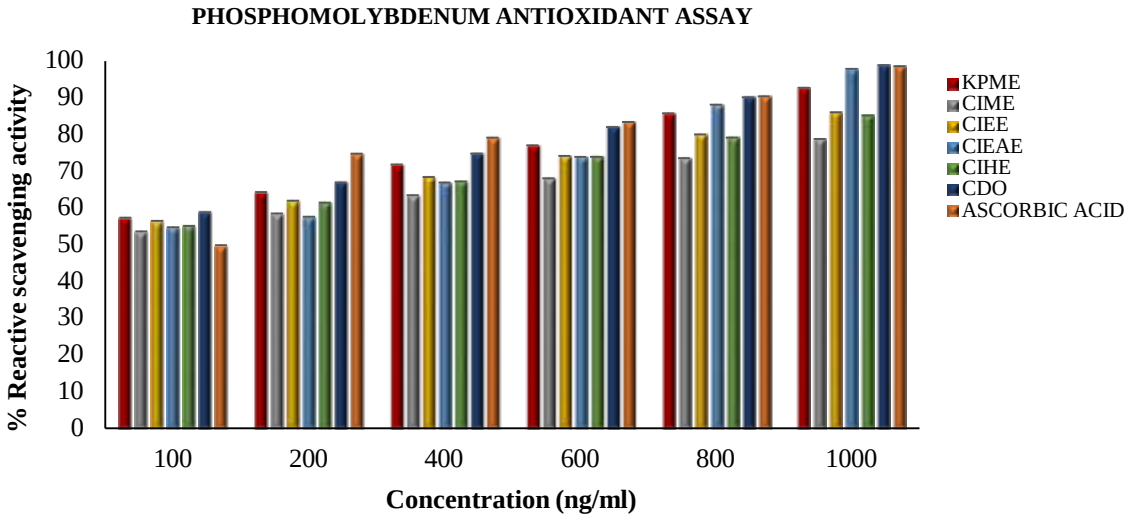

Figure 5. Phosphomolybdenum assay (Total antioxidant capacity) for determination of IC50 of plant extracts. Data illustrate as mean ± standard deviation (n = 3) at concentration of 100 -1000 ng/ml.

The obtained result of reducing power for distinct plant extract manifest higher IC50 value as compared to standard quercetin (46.23 ng/ml) and have antioxidant activity 99.48%. IC50 values for methanolic extract of *kigelia pinnata* fruit (430.73 ng/ml), ethyl acetate extract of *Cichorium intybus* root (671.82 ng/ml), ethanolic extract of *Cichorium intybus* root (342.14 ng/ml), hexane extract of *Cichorium intybus* root (238.97 ng/ml), methanolic extract of *Cichorium intybus* root (582.75ng/ml) and ethanolic extract of *Cedrous deodara* wood oil (464.91 ng/ml). Although free radical scavenging action found methanolic extract of the *kigelia pinnata* fruit (59.78%), ethyl acetate extract of *Cichorium intybus* root (62.5 %), ethanolic extract of *Cichorium intybus* root (65.76 %), hexane extract of *Cichorium intybus* root (71.875 %), methanolic extract of *Cichorium intybus* root (60.19 %) and ethanolic extract of *Cedrous deodara* wood oil (62.5 %). Reducing power method is mainly acclimated to determine extracts antioxidant activity to pledge an electron.[21] Most of the phenolic compounds have an antioxidant ability by donating the hydrogen.

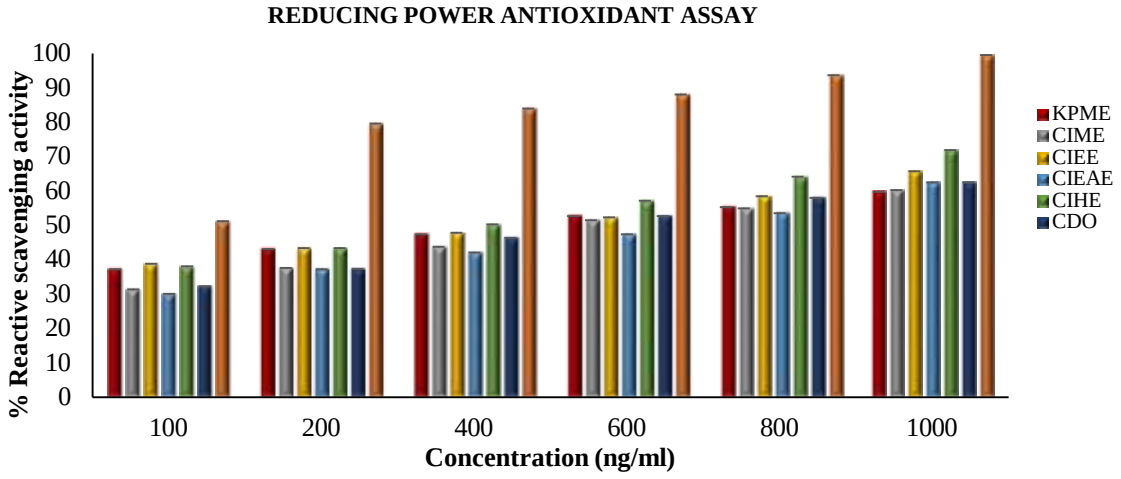

Figure 6. Reducing power antioxidant assay for determine IC50 of plant extract. Results illustrate as mean ± standard deviation (n = 3) at a concentration ranging from 100-1000 ng/ml.

Table 4. Identification of IC50 value from antioxidant assays

Sr No.	Phytoconstituent rich extract	DPPH Scavenging antioxidant activity (ng/ml)	Phosphomolybdate antioxidant activity (Total Antioxidant activity) (ng/ml)	Reducing power Activity (ng/ml)
1	KPME	1529.36	61.21	430.73
2	CIME	375.77	95.16	582.75
3	CIEE	358.43	71.00	342.14
4	CIEAE	304.51	64.44	671.82
5	CIHE	595.03	75.81	238.97
6	CDO	287.61	53.02	464.91
7	Ascorbic acid	57.37	56.22	-
8	Quercetin	-	-	46.23

Based on antioxidant studies (DPPH, Phosphomolybdate and reducing power) shows all extracts have ability to scavenge the free radical except significantly lesser in methanolic extract of *Kigelia pinnata* fruit as compared to standard ascorbic acid. There is no single phytoconstituent responsible for antioxidant activity. Phenolic, flavonoid, phenylpropanoids like compounds responsible for antioxidant activity by squeezing singlet and triplet oxygen, neutralizing free radicals may have potential for chelation of metals. Due to inclusion of numerous phytoconstituents, the extracts might possibly improve antioxidant properties in a synergistic manner.

4. CONCLUSION

Result obtained through qualitative analysis of different plant extracts by TLC method revealed presence of abundant phytoconstituents such as phenylpropanoids, iridoids, naphthaquinones, flavonoids, phenolics, terpenoids, sesquiterpenoids, sesquiterpene lactones, volatile oil, organic acid etc. Quantitative estimation of plant extract exhibit higher phenolic amount presence in methanolic extract of *C. intybus* root and *K. pinnata* fruit, root extracts of C. intybus in ethanol and methanol show more flavonoid content, terpenoid content elevated in *C. deodara* wood oil. High iridoids content was found only in methanolic extracts of *K. pinnata* fruit. This study also carried out for antioxidant potential of plant extracts by using DPPH, Phosphomolybdenum and reducing power. All plant extracts shows exemplary free radical scavenging activity in comparison with standard except methanolic extract have significant weaker. All the studied plant extracts showed lesser reducing power activity compared with quercetin. Thus, it is concluded from aforementioned study shows plant extracts exhibits prominent antioxidant ability can attribute to existence of some phenolic and Related phytoconstituents. As a result, it may be used as an antioxidant. In order to better understand its biological functions, further investigation is ongoing.

ACKNOWLEDGEMENT:
I heartily thankful to Dr. Alpna Shukla, Department of botany, M. G. Science Institute, Ahmedabad, Gujarat and Prof. Hitesh Solanki, Department of Botany, Gujarat University, Ahmedabad, Gujarat for authenticate Plant of *Kigelia pinnata* fruit and *Cedrous deodara* wood.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

ABBREVIATIONS: KPME – methanolic extract of *Kigelia pinnata* fruit, KPDCM- Dichloromethane extract of *Kigelia pinnata* fruit, CDO - *Cedrus deodara* wood oil (ethanolic extract), CIEE- Ethanolic extract of *Cichorium intybus* root, CIEAF- ethyl acetate extract of *Cichorium intybus* root, CIME- Methanolic extract of *Cichorium intybus* root, CIHE- Hexane extract of *Cichorium intybus* root, TPC-total phenolic content, TFC- total flavonoid content, TIC- total iridoid content, TTC- total terpenoid content

5. REFERENCES

1. Sharifi-Rad M, Anil Kumar N V., Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front Physiol. 2020;11(July):1–21.
2. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov. 2021;20(9):689–709.
3. Silva MM, Lidon FC. An overview on applications and side effects of antioxidant food additives. Emirates J Food Agric. 2016;28(12):823–32.
4. Bello I, Shehu MW, Musa M, Zaini Asmawi M,

Mahmud R. Kigelia africana (Lam.) Benth. (Sausage tree): Phytochemistry and pharmacological review of a quintessential African traditional medicinal plant. J Ethnopharmacol. 2016;189:253–76.
5. Nabatanzi A, Nkadimeng SM, Lall N, Kabasa JD, McGaw LJ. Ethnobotany, phytochemistry and pharmacological activity of kigelia africana (Lam.) benth. (bignoniaceae). Plants. 2020;9(6):1–29.
6. Janda K, Gutowska I, Geszke-Moritz M, Jakubczyk K. The common cichory (Cichorium intybus l.) as a source of extracts with health-promoting properties—a review. Molecules. 2021;26(6):1–14.
7. Das S, Vasudeva N, Sharma S. Cichorium intybus : A concise report on its ethnomedicinal, botanical, and phytopharmacological aspects . Drug Dev Ther. 2016;7(1):1.
8. Perović J, Tumbas Šaponjac V, Kojić J, Krulj J, Moreno DA, García-Viguera C, et al. Chicory (Cichorium intybus L.) as a food ingredient – Nutritional composition, bioactivity, safety, and health claims: A review. Food Chem. 2021;336(July 2020):127676.
9. Bisht A, Jain S, Misra A, Dwivedi J, Paliwal S, Sharma S. Cedrus deodara (Roxb. ex D.Don) G.Don: A review of traditional use, phytochemical composition and pharmacology. J Ethnopharmacol. 2021;279(November 2020):114361.
10. Article R. Gupta_et_al_2020.pdf. 2020;2(8):2010–20.
11. Medinipur P, Bengal W. Importance of Cedrus deodara (Roxb . Ex D . Don) G . Don : A review of its ethnobotany , phytochemical constituents and antioxidant potential Importance of Cedrus deodara (Roxb . Ex D . Don) G . Don : A review of its ethnobotany , phytochemical consti. Res Environ Life Sci 189. 2019;11(7):189–93.
12. Moideen SVK, Houghton PJ, Rock P, Croft SL, Aboagye-Nyame F. Activity of extracts and naphthoquinones from Kigelia pinnata against Trypanosoma brucei brucei and Trypanosoma brucei rhodesiense. Planta Med. 1999;65(6):536–40.
13. Nelisiwe Prenate Masuku, Sogolo Lucky Lebelo. Investigation of the Effects of Kigelia Africana (Lam.) Benth. Extracts on Tm3 Leydig Cells. Asian J Pharm Clin Res. 2019;12(10):87–92.
14. Epure A, Pârvu AE, Vlase L, Benedec D, Hanganu D, Gheldiu AM, et al. Phytochemical profile, antioxidant, cardioprotective and nephroprotective activity of romanian chicory extract. Plants. 2021;10(1):1–19.
15. Effects A inflammatory, Cichorium C, Roots L. Natural Product Communications. 2007;
16. Mohammad S, Khizar A, Faiza N, Mobasher A, Nawazish-i-Husain S, Fatima J, et al. Anticancer activity of n-hexane extract of Cichorium intybus on lymphoblastic leukemia cells (Jurkat cells). African J Plant Sci. 2014;8(6):315–9.
17. Loud R. Natural Product Communications Chemical Composition of Hydrodistilled and Solvent Volatiles Extracted from Woodchips of Himalayan Cedrus : 2009;9–12.
18. Total phenolic, flavonoid, alkaloid and iridoid contentand preventive effect of Lider-7-tang onlipopolysaccharide-induced acute lung injury in rats.pdf.
19. Łukowski A, Jagiełło R, Robakowski P, Adamczyk D, Karolewski P. Adaptation of a simple method to determine the total terpenoid content in needles of coniferous trees. Plant Sci. 2022;314.
20. Alvares JJ, Furtado IJ. Kinetics of DPPH• scavenging by bacterioruberin from Haloferax alexandrinus GUSF-1 (KF796625). J Anal Sci Technol. 2021;12(1).
21. Vasyliev GS, Vorobyova VI, Linyucheva O V. Evaluation of Reducing Ability and Antioxidant Activity of Fruit Pomace Extracts by Spectrophotometric and Electrochemical Methods. J Anal Methods Chem. 2020;2020.
22. Sadeer NB, Montesano D, Albrizio S, Zengin G, Mahomoodally MF. The versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations. Antioxidants. 2020;9(8):1–39.
23. Konuklugil B, Bahadir Ö. Phenylpropanoid glycosides from Linum olympicum (Linaceae). Turkish J Chem. 2004;28(6):741–4.
24. Wahab UHA, Awad ZJ. Isolation and Characterization of Iridoid Glycoside (Gardenoside ي) Present in the Leaves of Gardenia jasminoides J . ف ق اروا ت اب ن اي ن يدراك ال Ellis Cultivated in Iraq دي ودي رال ا دي ا سوك ي الك) دي ا سون يدراك ال (دوجومال .Sci عر س ت سم ال ي ف ق ارع ال ل صف ف ي صوت و .Iraqi J 2015;24(1):40–8.
25. Dzomba P, Mhini G. Isolation and Quantification of Flavonoids from Kigelia africana (Lam.) Benth Fruit and Bark and their Antiradical and Antibacterial Activities. Tanzania J Sci. 2021;47(1):57–69.
26. Pianaro A, Pinto JP, Ferreira DT, Ishikawa NK, Braz-Filho R. Iridóide glicosilado e derivados fenólicos antifúngicos isolados das raízes de Spathodea campanulata. Semin Ciências Agrárias. 2007;28(2):251.
27. Dinakaran SK, Chelle S, Avasarala H. Profiling and determination of phenolic compounds in poly herbal formulations and their comparative evaluation. J Tradit Complement Med. 2019;9(4):319–27.
28. Böhmdorfer S, Oberlerchner JT, Fuchs C, Rosenau T, Grausgruber H. Profiling and quantification of grain anthocyanins in purple pericarp×blue aleurone wheat crosses by high-performance thin-layer chromatography and densitometry. Plant Methods. 2018;14(1):1–15.
29. Prabhakaran P, Gopalakrishnan VK. Chromatographic fingerprint analysis of diterpenoids and sesquiterpenoids in n-hexane extract of Emilia sonchifolia (L.) DC by HPTLC technique. Int J

Pharm Pharm Sci. 2014;6(1):514–9.
30. van Beek TA, Maas P, de Groot A, King BM, Leclercq E, Voragen AGL. Bitter Sesquiterpene Lactones from Chicory Roots. J Agric Food Chem. 1990;38(4):1035–8.
31. Alam A, Majumdar RS, Alam P. Development of HPTLC method for determination of α-terpinyl acetate, and evaluation of antioxidant properties of essential oils in Elettaria cardamomum. Trop J Pharm Res. 2021;18(10):2139–45.
32. Bonfill M, Mangas S, Cusidó RM, Osuna L, Piñol MT, Palazón J. Identification of triterpenoid compounds of Centella asiatica by thin-layer chromatography and mass spectrometry. Biomed Chromatogr. 2006;20(2):151–3.
33. Wutsqa yu, suratman s, sari sla. Detection of terpenoids and steroids in Lindsaea obtusa with thin layer chromatography. Asian J Nat Prod Biochem. 2021;19(2):65–8.
34. Chaudhary A, Kaur P, Katiyar A, Singh B. HPTLC – Densitometry Method for Simultaneous Determination of Major Lignans and Flavonoids in Podophyllum hexandrum. 2012;25:314–9.
35. Douros A, Hadjipavlou-Litina D, Nikolaou K, Skaltsa H. The occurrence of flavonoids and related compounds in Cedrus brevifolia A. Henry ex Elwes & A. Henry needles. inhibitory potencies on lipoxygenase, linoleic acid lipid peroxidation and antioxidant activity. Plants. 2018;7(1):1–12.
36. Kruk J, Hassan B, Enein A, Duchnik E, Marchlewicz M. Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. J Physiol Sci. 2022;1–24.
37. Zeb A. Concept , mechanism , and applications of phenolic antioxidants in foods. 2020;(June):1–22.
38. Speisky H, Shahidi F, Camargo AC De. Revisiting the Oxidation of Flavonoids : Loss , Conservation or Enhancement of Their Antioxidant Properties. 2022;1–28.
39. Ling T, Boyd L, Rivas F. Triterpenoids as Reactive Oxygen Species Modulators of Cell Fate. 2022.
40. Habtemariam S. Iridoids and Other Monoterpenes in the Alzheimer ' s Brain : Recent Development and Future Prospects. 2018.