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Phytochemical Analysis and *In vitro* Anti-oxidant Activities of Medicinal Plants *Cyperus rotundus*, *Tinospora cordifolia* and their Formulation

S. GOMATHI[1,2] and S. MANEEMEGALAI[3]*

[1]Department of Biochemistry, Government Arts and Science College for Women, Orathanadu,-614625, Thanjavur DT, Tamil Nadu, India.
[2]Department of Biochemistry, Shrimati Indira Gandhi College (Affiliated to Bharathidasan University), Tiruchirappalli-620002, Tamil Nadu, India.
[3]*Biochemistry, Centre for Distance and Online Education, Bharathidasan University, Tiruchirappalli-620024, Tamil Nadu, India.
*Corresponding author E-mail: maneemegalai@gmail.com, drsmaneemegalai@bdu.ac.in

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ABSTRACT

Free radicals are harmful macromolecules that interact with lipids, nucleic acids and carbohydrates inside cells. The high level of ROS present in the cell leads to major health issues such as diabetes, hypertension, cancer and oxidative stress. In the current study, *Cyperus rotundus (C.rotundus)*, *Tinospora cordifolia (T.cordifolia)* and their formulations were evaluated for their phytochemical content, functional group identification by FT-IR and *In vitro* free radical scavenging activity. The phytochemical analysis showed that the ethanolic extracts had more secondary metabolites like flavonoids, alkaloids, terpenoids, and phenolic compounds than the extracts from the other solvents. Compared to the standard ascorbic acid, the ethanolic extract exhibited good free radical scavenging activity against DPPH, ABTS, hydrogen peroxide and superoxide and an elevated level of activity was observed with the increased concentration of the extract. The ethanolic extracts of *C.rotundus*, *T.cordifolia* and their formulation were investigated using FT-IR spectroscopy, which revealed the presence of unique functional groups such as primary as well as secondary amines, alkenes, nitro compounds and alkylhalides. In an *In vitro* model, the current finding demonstrates the anti-oxidant effectiveness of *C.rotundus* and *T.cordifolia*. Hence, further studies are warranted and it might eventually be utilized as a possible medicament for treating diseases.

Keywords: Anti-oxidant, Phytochemical analysis, FT-IR analysis, *Cyperus rotundus*, *Tinospora cordifolia*.

INTRODUCTION

Oxidative stress results in the production of large amounts of highly reactive oxygen species, which cause DNA damage. The best-known free radicals that are highly toxic to cells are hydroxyl ions, hydrogen peroxide, superoxide, nitric oxide and oxides of nitrogen. There are many possible medicines available in nature. The World Health Organization states that drugs derived from plants are used in 80% of all traditional medical procedures worldwide. Indian traditional medical systems have mostly used the pharmacological analysis of several therapeutic plants[1]. Natural bioactive substances

known as "antioxidants" prevent oxidation by scavenging free radicals. Essential bioactive secondary metabolites produced by medicinal plants, such as alkaloids, phenols, and flavonoids, can be used to treat a number of fatal degenerative diseases, notably cancer, cardiac issues and neurological disorders caused by oxidative stress[2].

Generally two kinds of primary and secondary anti-oxidants are available the primary antioxidant directly scavenging the free radicals and secondary antioxidant are indirectly impede the free radical production via fenton's reaction. Plant extracts and natural products are plays primary anti-oxidant role by scavenging free radicals. Antioxidants are applicable in many fields such as food additives to protect food deterioration, as stabilizers for lubricants and fuels to delay/prevent from oxidation, in gasolines to protect polymerization which leads to engine-fouling residue formations, as drug for cancer, asthma, diabetes, inflammatory joint diseases, atherosclerosis, degenerative eye disorders and senile dementia.

Cyperus rotundus, a member of the Cyperaceae family, can be found in tropical, subtropical, and temperate climates. It is commonly known as motha, purple nutsedge or nutgrass, Korai kizhangu in Tamil language[3]. One of the useful medicinal plant parts used to cure nausea, diarrhoea, fever and inflammation is the rhizome of *C. rotundus*[4]. It is a hazy medicinal herb that has been connected to an extensive range of effects, like analgesia, antibacterial activity, antidiarrheal activity, antidiabetic activity, anti-inflammatory activity[5], antioxidant activity, appetiser, digestive and sedative effects[6,7].

Tinospora cordifolia, also known as guduchi and "Shindilakodi," in Tamil, is a member of the Menispermaceae family and is found all across India. It is a tall, climbing shrub with broad leaves. It has a number of health benefits, such as reducing inflammation, protecting the liver, boosting the immune system, lowering blood sugar and fighting cancer[8,9]. So, the aim of recent research is to find out whether *C. rotundus* and *T. cordifolia* extracts work as antioxidants.

MATERIAL AND METHODS

Collection and authentication of plant source

The rhizome part of *C. rotundus* and stem part of *T. cordifolia* were collected from the region of Lalgudi area, Tiruchirappalli district, Tamil Nadu, India. The obtained plant samples were verified as real, and voucher specimens were placed at the Rapinat Herbarium, located at St. Joseph's College (autonomous), Tiruchirappalli, Tamil Nadu, India. The materials were collected, distilled water rinsed thrice and shade dried at room temperature. The pure plant materials were crushed into a coarse powder for further research.

Preparation of plant extract

The soxhlet hot extraction method was used to extract the phytocompound from the dried powder of *C. rotundus* and *T. cordifolia* using different organic solvents of water, ethanol, chloroform, ethyl acetate and petroleum ether. The different solvent extracts were made separately, for the plants *C. rotundus* and *T. cordifolia* as well as the combination of both plants (1:1 ratio).

Phytochemical screening

Different extracts of *C. rotundus* and *T. cordifolia* and its formulations were qualitatively screened for the phytoconstituents present[10].

Estimation of total phenolic and flavonoid content

Using Folin-ciocalteu method, the total phenolic content of *C. rotundus* and stem of *T. cordifolia*, its formulations were measured[11]. Similarly, using the aluminium chloride method, the total flavonoid content of C*. rotundus and T. cordifolia,* as well as their formulations, was determined[12].

In vitro antioxidant assay

On the basis of a single antioxidant assay, the antioxidant activity of the plant extract should not be determined. In order to assess antioxidant activity, several *In vitro* test techniques such as the DPPH radical scavenging assay, ABTS radical scavenging activity, hydrogen peroxide scavenging activity, superoxide radical scavenging assay, and total anti-oxidant assay method are used. The extracts of *C.rotundus* and *T.cordifolia* and their formulations were tested against free radical scavenging properties. It determines the extract's potential to neutralise the free radicals and the change in optical density of radicals is observed in order to assess the antioxidant capability through free radical scavenging by the plant extract.[13, 14, 15, 16,17.] The scavenging effect was calculated by using the formulae:

Scavenging effect (%)=(Absorbance of control–Absorbance of test solution or Standard)/Absorbance of control]×100

Fourier transform infrared spectrophotometer (FT-IR) analysis

To prepare the sample disc for FT-IR spectroscopy, 10 mg of the sample was encapsulated in 100 mg of potassium bromide pellets[18]. The extract's functional groups absorbed light with a wavelength between 4000 and 400 cm^{-1}.

RESULTS

Phytochemical screening

A preliminary phytochemical analysis was done on the rhizome of *C. rotundus*, the stem extract of *T. cordifolia*, and their mixtures. This result showed that phytochemicals were present in aqueous, ethanol, chloroform, ethyl acetate, and petroleum ether extracts. These phytochemicals include flavonoids, diterpenes, proteins, saponins, amino acids and sugars. (Table 1). The phytochemical analysis of the *C. rotundus* rhizome revealed that the ethanolic and aqueous extracts had the highest amounts of various phytochemicals like alkaloids, flavonoids, steroids, tannins, phenols, and saponins. Steroids, phenol and petroleum ether are also present in the chloroform, ethyl acetate, and petroleum ether extracts of *C. rotundus.*

Phytochemical analysis of the stem of *T. cordifolia* revealed the presence of alkaloids, flavonoids, steroids, tannins, phenols and saponins in the aqueous and ethanolic extracts. Quinine, coumarins and glycosides were also present in the ethyl acetate and petroleum ether extracts of *T. cordifolia*. Alkaloids, flavonoids, steroids, tannins, quinine, coumarins, saponins and phenols were present in the formulations of *C. rotundus* and *T. cordifolia*. Tannin, quinine and phenols were more abundant in the aqueous and ethanolic extracts than the other three extracts studied. The ethanolic extract of *C. rotundus* and *T. cordifolia* were found to contain most of the phytochemicals.

Alkaloids, flavonoids, steroids, tannins, phenols, and saponins were found in high amounts in the stem extracts of *T. cordifolia* (both aqueous and ethanol). Quinine, coumarins, and glycosides were also found in the extracts of *T. cordifolia* in ethyl acetate and petroleum ether. The mixtures of *C. rotundus* and *T. cordifolia* had phenols, tannins, quinine, coumarins, flavonoids, alkaloids, and steroids. The aqueous and ethanolic extracts showed higher levels of tannin, quinine, and phenols when compared to other three extracts. The aqueous and ethanolic extracts of *C. rotundus* and *T. cordifolia* were found to contain the majority of the phytochemicals.

Total phenolic content

The estimated phenolic content was expressed as gallic acid equivalent (GAE) per gram of plant extract Fig. 1. The highest concentration of total phenolic contents was observed 85.14±7.82 mg GAE/g of dry extract in the formulation than the individual plant extract (58.27±5.24, 45.43± 6.24 mg GAE/g of dry extract Table 2.

Total flavonoids content

Total flavonoid content was expressed as quercetin equivalent (QE) per gram of plant extract Fig. 2. The total flavonoid contents of *C. rotundus* and *T. cordifolia* extracts were found to be 110±9.52 mg QE/g of dry extracts, respectively compared to the individual plant extracts (90.70±8.96, 56.43±6.94) in Table 2.

Table 1: Phytochemical screening of *C. rotundus* and *T. cordifolia* and their formulations

S. No	Phytochemicals	*C. rotundus*					T. cardifolia					*C. rotundus* & T. cardifolia				
		A	E	C	EA	P	A	E	C	EA	P	A	E	C	EA	P
1	Alkaloids	-	+++	-	++	++	+++	+++	-	++	-	+++	+++	-	++	-
2	Flavonoids	+++	+	-	-	-	-	+++	-	++	-	+++	+++	-	-	-
3	Steroids	-	+++	+	++	++	+++	-	-	-	+	+++	+++	-	-	-
4	Tannim	+++	+++	-	-	++	+++	+++	-	-	-	+++	+++	++	-	++
5	Terpenoids	+++	+++	-	-	-	-	-	-	-	-	-	+++	-	-	-
6	Quinine	+++	-	-	++	-	-	+++	-	++	++	+++	+++	++	++	++
7	Coumarine	+++	+++	-	-	-	+++	+++	-	++	-	+++	+++	-	-	-
8	Gtyconides	+++	+++	-	-	-	+++	+++	++	++	++	-	-	-	-	-
9	Saponins	+++	+++	-	-	-	+++	-	-	-	-	+++	+++	-	-	++
10	Phenols	+++	+++	+	++	++	-	+++	++	+++	-	+++	+++	++	++	++

Abbreviation: A-Aqueous, E-Ethanol, C-Chioroform, EA-Ethyl acetate, P-Petroleum Ether (+++)-High abundant, ++moderate abundant,+abundant, (-)negative

Table 2: Total Phenolic and Total flavonoid content of plant extracts

Extract	Total phenolic content (expressed as Gallic acid equivalent (GAE) per gram of plant Extract)	Total flavonoid content expressed as (Quercetin equivalent (QE) per gram of plant Extract)
C.rotundus	58.27±5.24	90.70±8.96
T. cardifolia	45.43±6.24	56.43±6.94
C. rotundus & T. cardifolia	85.14±7.82	110±9.5

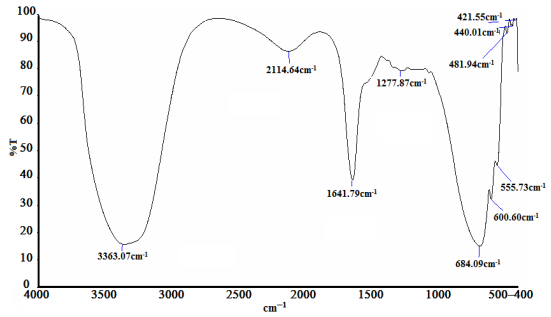

Fig. 1. FT-IR analysis of *C. rotundus*

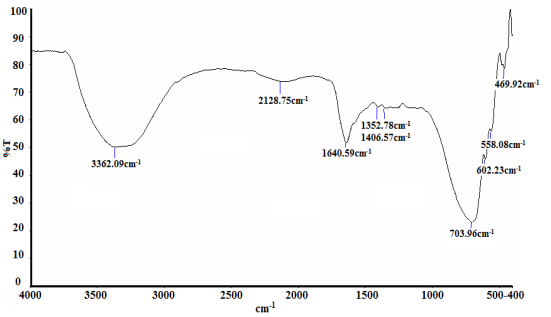

Fig. 2. FT-IR analysis of *T. cordifolia*

DPPH free radical Scavenging Assay

This study also established the IC_{50} values for the different concentrations of plant extract required to scavenge 50% of the DPPH radicals. The IC_{50} values for the extracts of *C. rotundus* and *T. cordifolia* combined were 250 µg/mL, 300 µg/mL, and 250 µg/mL, respectively. Nevertheless, conventional ascorbic acid has an IC_{50} value of 250µg /mL (Table 3).

ABTS radical scavenging activity

At 300 g/mL, the highest percentage of inhibitory activity for *C. rotundus* was 58.68%, and the highest percentage for *T. cordifolia* was 55.97%. The combination of plant extracts has the highest activity (54.9%) at 250 µg/mL concentration, and the activity of conventional ascorbic acid is 57.14% at 300 µg /mL. These outcomes showed the potential activity of a plant extract formulation (Table 4).

Table 3: DPPH radical scavenging activity of *C. rotundus*, *T.cordifolia* and its formulations

Con. (µg/mL)	% of Inhibition *C.rotundus*	% of Inhibition *T. cordifolia*	% of Inhibition *C.rotundus* & *T. cordifolia*	Standard Ascorbic acid
50	12.90± 0.003	10.77±0.128	15.75±0.218	12.23±0.116
100	20.03±0.125	19.17±0.009	24.42±0.241	21.63±0.104
150	31.37±0.007	28.78±0.165	36.26±0.330	34.41±0.234
200	40.36±0.125	37.99±0.214	46.30±0.561	44.49±0.486
250	50.18±0.413	47.75±0.416	56.75±0.431	54.31±0.452
300	59.95±0.312	58.74±0.428	65.56±0.543	60.29±0.375
350	67.08±0.413	64.79±0.585	73.86±0.654	67.00±0.621
IC_{50} value	250.285	263.753	233.283	227.490

Table 4: ABTS radical scavenging activity of *C. rotundus*, *T. cordifolia* and its formulations

Con. the Sample (µg/mL)	% of Inhibition *C. rotundus*	% of Inhibition *T. cordifolia*	% of Inhibition *C. rotundus* & *T. cordifolia*	Ascorbic acid
50	13.62 ± 0.021	13.31± 0.020	17.5 ± 0.018	13.88 ± 0.021
100	16.47 ± 0.052	17.78 ± 0.047	24.2 ± 0.021	15.85 ± 0.031
150	23.77 ± 0.327	25.90 ± 0.031	35.2 ± 0.036	27.85 ± 0.034
200	38.09 ± 0.361	34.66 ± 0.030	42.9 ± 0.031	35.44 ± 0.029
250	44.84 ±0.024	44.32 ± 0.029	55.9 ± 0.025	45.06 ± 0.027
300	58.68 ±0.037	55.97 ± 0.032	64.6 ± 0.038	57.14 ± 0.037
350	68.65 ±0.035	60.71 ± 0.026	76.0 ± 0.027	63.97 ± 0.031
IC_{50} value	284.67	294.52	227.80	286.57

Hydrogen peroxide scavenging activity (H_2O_2)

The activity of plant extracts on hydrogen peroxide scavenging assay were found to be maximum in the combination of plant extract (248.91±25 µg/mL).The results revealed that the ethanolic extracts of *C. rotundus* and *T. cordifolia* have moderate scavenging activity (61.60±5.78%, 58.86±4.32%), at a high concentration of 350 µg/mL. The combination of herbal extracts (85%) had more potent hydrogen peroxide scavenging activity than the standard reference of ascorbic acid (Table 5).

Superoxide radical scavenging activity

The results suggested that the ethanolic extract of *C. rotundus*, *T. cordifolia* and their combinations were greater scavengers of superoxide with a value of 55.28±4.3%, 55.17±4.72% and 57.76±5.324% at a concentration of 250 µg/mL compared to the ethanolic extract of *C. rotundus, T. cordifolia* at the same concentration (Table 6).

Total antioxidant activity

Total antioxidant activity of ethanolic extract of *C. rotundus*, *T. cordifolia* and its formulations was carried out by phosphomolybdenum method (Table 7). The total antioxidant activity is expressed as equivalent to ascorbic acid. The results indicated the dose dependent increase in antioxidant activity at concentration 50 to 350 µg/mL. The strong antioxidant activity of combinations of these plant extracts was higher 93.34% at 350 µg/mL. The individual plant extracts of *C. rotundus, T. cordifolia* exhibited 89.91% at 350 µg/mL, 90.45% at 350 µg/mL respectively. The presence high flavonoid and phenolic contents might be the reason for the antioxidant capacity of medicinal plants.

Table 5: Hydrogen Peroxide scavenging activity of *C.rotundus, T.cordifolia* and its formulations

Con. (µg/mL)	% of Inhibition *C. rotunus*	% of Inhibition *T. cordifolia*	% of Inhibition *C. rotundus* & *T. cordifolia*	Ascorbic acid
50	13.57 ± 0.021	13.27 ± 0.138	13.72 ± 0.321	13.29± 0.021
100	14.61 ± 0.352	14.48 ± 0.216	21.42 ± 0.392	14.58± 0.234
150	23.88 ± 0.427	21.50 ±0.354	29.71 ± 0.472	24.92± 0.354
200	32.95 ± 0.261	31.38 ± 0.482	39.91 ± 0.257	31.50± 0.432
250	43.81 ± 0.372	42.8 ± 0.672	51.74 ± 0.484	49.03 ±0.237
300	51.69 ±0.432	48.9 ±0.457	60.18 ± 0.419	48.86 ±0.413
350	61.60 ±0.278	58.86 ±0.432	68.64 ± 0.356	65.60 ±0.285
IC_{50} value	303.743	316.627	248.91	281.991

Table 6: Superoxide radical scavenging activity of *C. rotundus*, *T. cordifolia* and its formulations

Con. of the Sample (µg/mL)	% of Inhibition C.rotundus	% of Inhibition T. cordifolia	%of Inhibition C.rotundus & T.Cordifolia	Ascorbic acid
50	19.46 ± 0.021	20.22± 0.038	23.97 ±0.026	21.86± 0.023
100	27.83± 0.052	30.01± 0.016	31.75 ± 0.192	26.87± 0.354
150	39.54± 0.327	40.43±0.254	42.42 ± 0.172	41.29±0.218
200	46.40± 0.361	46.35± 0.382	48.59 ± 0.257	47.73± 0.432
250	55.28±0.243	55.17±0.472	57.76 ± 0.324	56.61±0.340
300	64.82±0.0375	62.11±0.371	65.29±0.276	64.2 ±0.410
350	68.65±0.256	68.96±0.287	70.68 ± 0.327	69.21 ±0.347
IC_{50} value	218.163	217.045	203.909	211.576

Table 7: Total antioxidant activity of C.rotundus and T.cordifolia and its formulations

Con. (µg/mL)	% of *C. rotundus*	% *T. cordifolia*	% of *C. rotundus* & *T. cordifolia*	% of Ascorbic acid
50	30.18	24.18	40.18	30.35
100	40.17	40.13	50.02	42.23
150	50.02	51.14	65.17	52.18
200	61.18	64.19	75.19	65.30
250	69.98	70.64	80.14	70.16
300	70.04	82.35	90.05	80.06
350	89.91	90.45	93.34	85.15

FT-IR analysis of *C. rotundus*

FT-IR analysis was done on the ethanolic extract of *C. rotundus* to determine the potential biomolecules. *C. rotundus* has a lot of absorption bands, which means it has a lot of functional groups. While some intensity peaks, like those for the periods 1638 cm^{-1} and 684 cm^{-1} are decreased, others, like those for the periods 3363 cm^{-1}, 2114 cm^{-1}, 1641 cm^{-1} and 1277 cm^{-1} are greatly enhanced. The band at 3363, as shown in Fig. 1, depicts N-H1, the stretching vibrations of primary, secondary, and tertiary amines and amides. At 2114 cm^{-1}, the C-N Stretch in plane bend to Nitriles was depicted. The peak at cm^{-1} is caused by the C=O stretching vibrations to carbonyls. The presence of alkyl halides in the *C. rotundus* extract is consistent with the faint band at 684, which denotes CBr Stretch vibrations.

FT-IR analysis of *T. cordifolia*

The graph showed the ethanolic extract of *T. cordifolia* peak value and functional groups from the FT-IR spectrum. Strong intensity peaks at 3362 cm^{-1} and 2128 cm^{-1} were visible in this spectra, as well as smaller peaks at 1640 cm^{-1} and 558 cm^{-1}, which respectively denote the presence of primary amines as well as secondary amines, nitriles, alkenes, nitro compounds, and alkyl halides Figure 2.

FT-IR analysis of *C. rotundus* and *T. cordifolia*

Figure 3 displays the FT-IR spectrum's results. The large, noticeable peak at 3369 cm^{-1} and 2126 cm^{-1}, which correlates to the presence of alkene compounds, is illustrated in this spectrum of formulations of *C. rotundus* and *T. cordifolia* (Fig. 3). The signal at 1642 cm^{-1} similarly denotes the presence of nitro compounds. The faint band at 668 cm^{-1} indicates that alkynes are present in the formulation.

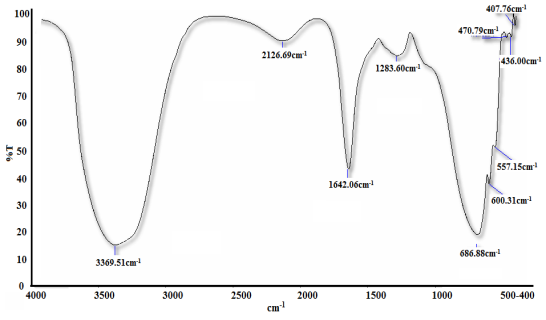

Fig. 3. FT-IR analysis of *C. rotundus* and *T. cordifolia* formulation

DISCUSSION

Plants contain naturally occurring substances known as "phytochemicals." Due to their antioxidant or disease-preventing actions, the secondary metabolites, such as alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates and phenolic compounds, have numerous therapeutic capabilities, such as in the instances of heart disease, cancer, hypertension and diabetes[19]. Alkaloids act as analgesic and antispasmodic agents, while saponins have antimicrobial and antifungal properties. The bioactive compounds namely alkaloids, saponins, flavonoids and phenolic compounds acts as a defense mechanism in humans to inhibit degenerative diseases and has pharmacological activity, which includes free radical scavenging activity, anti-inflammatory activity, and cytotoxicity[20].

Phenolic compounds and flavonoids like flavones and flavanols have redox potentials that enable them to provide free radicals with hydrogen atoms; they act as antioxidants. These substances are crucial for the prevention of illnesses connected to oxidative stress[21]. The observations of this investigation demonstrated that the high levels of polyphenols and flavonoids in the ethanolic extracts of *C. rotundus* and *T. cordifolia* gave rise to the highest antioxidant capacity[22].

Reactive oxygen species are produced as a result of biological metabolism, are the most prevalent reactive oxygen species are superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals[23]. Reactive species may be causing DNA mutations that result in tissue damage, cancer, and neurogenic illness through oxidation. Moreover, it performs a number of significant activities, including suppressing tumors, delaying cell damage and enhancing cellular defenses against different diseases. Antioxidants protect cells from the ailment inducing effects of free radicals[24]. The DPPH, superoxide radical scavenging assay, hydrogen peroxide scavenging activity and total antioxidant activity were used in the current investigation to measure the *In vitro* antioxidant activity of plant extract.

The most commonly used method for determining a plant's capacity for antioxidants is the DPPH radical scavenging assay. *In vitro* DPPH radical activity of the ethanolic extracts of *C. rotundus*

and *T. cordifolia* was evaluated. The present results suggest that plant extracts' phytochemicals have the ability to scavenge free radicals and lower the risk of oxidative diseases. The previous research explained the antioxidant activity of plant extract by measuring the ability to bring down free radicals and expressed in terms of hydrogen atom donating ability[25].

An important and widely used technique to assess the antioxidant capacity of plant extracts is the ABTS free radical scavenging assay. The antioxidant effect of a plant extract needs ABTS to lose its color, which also makes free radical species ineffective. At 734nm, the average absorbance was measured, which made it harder for proton radicals to get rid of extra electrons. These findings demonstrated that formulations of *C. rotundus* and *T. cordifolia* had effective radical scavenging effects against ABTS free radicals free radical ABTS effects. The previous study reported the dose dependent increase in the *In vitro* scavenging activities of methanolic extracts of *Caesalpinia volkensii* Harms. *Vernonia lasiopusO*. Hoffm., and *Acacia hockii De* Wild[26].

A weak oxidizing agent, hydrogen peroxide, instantly inactivates the enzyme. A reactive free radical formed in the biological system can easily cross the cell membrane. The generation of hydroxyl radicals interacts with transition metal ions like Fe^{2+} and Cu^{2+} to severely damage cells[27]. The interaction of antioxidant chemicals with hydrogen peroxide neutralizes it. In comparison to the ascorbic acid standard reference, the mixture of *C. rotundus* and *T. cordifolia* (85%) demonstrated strong hydrogen peroxide scavenging action. Similarly previous study reported the higher potency free radical scavenging activity of different leaf extracts from *Kedrostis foetidissima* (Jacq.)[28].

Superoxide anion is harmful free radical to the cellular components produced by the incomplete metabolism of oxygen. The inefficient use of oxygen in the body makes superoxide anion, which is a dangerous free radical for biological parts. As a result, it also causes harm to biomolecules by causing the production of H_20_2, OH, peroxynitrite, or singlet oxygen. To keep superoxide radicals from hurting the cells, they must be neutralized[29]. Due to the presence of flavonoids, the study's findings showed that the mixture of plant extracts had excellent potential for creating superoxide radicals. Recently, research reported that the crude extract of solanum nigrum linn shows high degree of super oxide radical activity[30].

Poly phenolic like flavonoids, phenolic acids and tannins are regarded as powerful antioxidant of plant extract. These compounds are considered as reducing agents and free radical quenchers that various medicinal properties like antibacterial, antiviral and antidiabetic activities[31]. In our study, the ethanolic extract of formulation of plant extract exhibited high antioxidant potential compared to the reference standard. Scavenging potential of the plant extract also depends on the high content of natural polyphenol compounds present in the plant extract[32].

The most important method for identifying the functional groups of the bioactive components found in the plant extract is FT-IR spectroscopic analysis. The ethanolic extracts of *C. rotundus* and *T. cordifolia* contained alkenes, alcohols, alkanes, alkyl halides, and aldehydes, according to the findings of FT-IR analysis[23].

CONCLUSION

From the overall observations, it is to state that the ethanolic extracts of *C. rotundus* and *T. cordifolia* exhibited the maximum free radical scavenging activities in a synergistic way in an in vitro model. Hence, further researches are required to explore the therapeutic value of *Cyperus rotundus* and *Tinospora cordifolia*.

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Conflict of interest

The author declare that we have no conflict of interest.

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