Comparative Phytochemical Screening and Antioxidant activity of *Actinidia Deliciosa* and *Ocimum Gratissimum* Leaves extract and combined mixture of both plants extracts using successive extraction methods.

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Abstract

Actinidia deliciosa L., commonly known as kiwifruit, belongs to the *Actinidia* genus within the *Actinidiaceae* family. The fruits of these plants are known to possess various beneficial properties such as anti-diabetic, antioxidant, anti-inflammatory, antifungal, anticarcinogenic, hepatoprotective, and antimicrobial activities. *Ocimum gratissimum*, on the other hand, is an herbaceous plant that belongs to the *Lamiaceae* family, formerly known as *Labiatae*, commonly referred to as "cloves basil" and is recognized for its aromatic leaves and potential medicinal properties. In traditional medicine, it's commonly used for treating a range of issues like upper respiratory tract infections, diarrhea, headaches, conjunctivitis, skin conditions, pneumonia, tooth and gum problems, fever, and as a mosquito repellent. In this research focused on the comparative study of phytochemical constituents of both plant and in vitro study the antioxidant property characterized by using 2,2`-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging ativity, of the plant leaves extract, of *Actinidia delicosa* and *Ocimum gratissimum*, using successive extraction method, and also evaluates the antioxidant property of the mixture of their extracts.

Key words: *Actinidia deliciosa L, Ocimum gratissimum,* Antioxidant, successive extraction, 2,2`-diphenyl-1-picrylhydrazyl radical (DPPH),

1. Introduction:

Free radicals are waste substances produced by cells. If the body cannot process and remove free radicals efficiently, oxidative stress can result, this can harm cells and body function[1]. Oxidative stress is characterized by the occurrence of oxidative damage due to an unfavorable imbalance between the generation of free radicals and the body's antioxidant defenses. This condition, resulting from an unequal relationship between the production of free radicals and the protection provided by antioxidants, is linked to the harm inflicted upon various molecular components such as lipids, proteins, and nucleic acids. Many other effects of the oxidative stress resulting from free radicals to a range of health conditions, including neurological disorders like Alzheimer's and various forms of dementia, cardiovascular diseases attributed to arterial blockages, autoimmune and inflammatory disorders such as rheumatoid arthritis and cancer, cataracts and the decline of vision associated with aging, aging-related alterations in appearance, encompassing diminished skin elasticity, wrinkles, graying hair, hair loss, and shifts in hair texture, diabetes, genetic degenerative diseases like Huntington's disease or Parkinson's.[2]

Antioxidants play a crucial role in maintaining the balance within our bodies. These molecules act as defenders, neutralizing free radicals that, when present in excess, can contribute to various illnesses like diabetes, heart disease, and cancer[3].

While the body possesses its own antioxidant defenses, obtaining antioxidants from dietary sources is essential. Fruits, vegetables, and other plant-based whole foods are rich in antioxidants, with vitamins E and C being notable examples. Consuming a diverse range of these foods can contribute to bolstering the body' defenses against oxidative stress[4]. Additionally, in the realm of food production, antioxidant preservatives serve a vital function by extending the shelf life of products. This dual role highlights the significance of antioxidants both in promoting human health and ensuring the longevity of food items[5].

The *Actinidia deliciosa* is a deciduous and woody fruiting vine, well known as kiwi fruit classified under the *Actinidia* genus family *Actinidiaceae*. Abundant in ascorbic acid and polyphenolic compounds, the fruit possesses antioxidant properties that contribute to lowering the risk of cardiovascular disease, arteriosclerosis, various cancers, and providing other health benefits[6]. In traditional medicine, kiwifruits are employed for treating diverse malignancies, such as stomach, lung, and liver cancer. It stands out as a globally renowned fruit, renowned for its diverse biological properties, encompassing antioxidant, anti-allergic, and cardiovascular protective effects. The peel of kiwi fruit, often overlooked during processing, emerges as a valuable reservoir of flavonoids[7].

*Ocimum gratissimum (*Linn), an herbaceous plant from the Labiatae family, popularly known as scent leaf, clove basil and nimma tulasi, is native to tropical regions, particularly India, and is also present in West Africa[8]. It thrives in both savannah and coastal regions. Traditional medicine utilizes this plant to address various ailments, such as upper respiratory tract infections, diarrhea, headaches, conjunctivitis, skin diseases, pneumonia, tooth and gum disorders, fever, and as an effective mosquito repellent[9]. Numerous phytochemicals, including flavonoids, polyphenols, and volatile substances including geraniol, thymol, and eugenol, are present in *Ocimum gratissimum*. Vasorelaxation, anti-inflammatory, anti-mycotoxigenic, anti-fungal, anti- microbial and antioxidant properties are some of its medical applications[10].

In this work the leaves of *Actinidia deliciosa,* which is an agricultural waste product, and the leave of *Ocimum gratissimum* was collected and undergo extraction of phytochemicals an perform the comparative study of antioxidant activity using DPPH radical scavenging model, and also the mixture of their extract.

2. Materials And Method:

2.1. Collection and Authentication of Plant:

Fresh ***Actinidia deliciosa*** leaves free of disease, was collected from **Jakholi block in Rudraprayag district, Uttarakhand, India** about 2,936 feet above sea level and the ***Ocimum gratissimum*** leaves free of disease, was collected from **Khusro Bagh, Lukarganj in Prayagraj, Uttar Pradesh**, **India**. The plant material was authenticated and certificated, at the Botanical Survey of India, Central Regional Centre, located at 10 Chatham Lines, Prayagraj, 212002.

2.2. Chemicals and Reagents Used :

Ethanol, n-hexene, ethyl acetate, methanol, dragendorff's reagent, lead acetate, ferric chloride, sodium hydroxide, hydrochloric acid, sulphuric acid, chloroform, silica gel-G, acetone, mayer's reagent, Ninhydrin, wagner's reagent, a-naphthol, 2,2`-diphenyl-1-picrylhydrazyl radical (DPPH), ascorbic acid.

2.3. Preparation of Plant extract :

The leaves were cleaned twice: once with sterilized water and once with running tap water. In a dimly light room, the leaves were shade-dried. After being dried, was grinded into a fine powder and kept in an airtight glass container until needed.
Using the Soxhlet extraction technique, a successive extraction process is prepared by using different solvents starting from the non-polar to the polar and hydroalcholic solvent[11].

2.4. Photochemical Screening:

The qualitative test was performed to identify the presence of the phytochemical such as alkaloids, flavonoid, phenols, amino acids, glycosides in both *Actinidia deliciosa* and *Ocimum gratissimum* plant extracts.

2.5. Thin Layer Chromatography analysis :

The TLC plate is been prepared fallowing the standard procedure, and the mobile phase formed by the mixture of n-hexane and ethyl acetate at the ratio of 1:1 in the developing chamber. The spots of the extract were placed on the TLC plate and after that place the plate in the developing chamber[12].

2.6. High performance thin layer chromatography analysis :

HPTLC fingerprint profiling was used to identify flavonoids in the ethyl acetate and hydroalcoholic extract of *Actinidia deliciosa* leaves, with quercetin serving as a reference marker. To get the best separation and clear peaks, a variety of solvent solutions were examined. 1 milligram of quercetin was dissolved in 1 milliliter of methanol to produce a stock solution, which yielded 1 mg/ml or 1000 ppm of the chemical. This stock solution was prepared into four dilutions using methanol: 50, 100, 150, and 200 ppm dilutions. A 5:4:1 mixture of toluene, ethyl acetate, and formic acid was used as the ideal solvent solution for the mobile phase.
To do the quantification, methanol was added to the hydroalcoholic and ethyl acetate extracts of *Actinidia deliciosa* leaves at a concentration of 1 mg/ml. To get a 150 ppm concentration, a dilution of these extracts was made. TLC was performed using pre-coated silica gel HPTLC aluminum plates (10 cm × 10 cm, 0.2 mm thick). The two leaf extracts in 16 µl quantities were detected as 6 mm broad bands using an auto sampler equipped with a 100 µl Hamilton syringe. Twenty milliliters of the mobile phase were added to the solvent system to pre-saturate it after it had been transferred to a development chamber. The resulting plates were scanned once they had dried. Process findings, record spectra, and use a spectrodensitometer. The absorption/remission measurement was carried out at a scan speed of 20 mm/s. Chromatograms were obtained at wavelengths ranging from 200 to 510 nm. The Rf value of each chemical was recorded.

2.7. Antioxidant activity :

The sample of the plant extract was collected and coded according to the Table 1The stable free radical DPPH (2,2`-diphenyl-1-picrylhydrazyl) radical has been frequently used for its substantial antioxidant activity. Following an electron or hydrogen radical agreement, DPPH is transformed into the stable DPPH-H structure. The rich violet color of DPPH becomes yellow when this conversion takes place. A

UV spectrophotometer detects unconverted DPPH at 517 nm. 3.0 milliliters of the sample were mixed with 1.0 milliliter of the 0.1 mM DPPH in ethanol solution. The absorbance was measured at 517 nm after 30 minutes. The standard substance used was ascorbic acid.
Significant free radical-scavenging activity was shown by the reaction mixture's decreased absorbance. Every test was carried out three times. The following equation was used to study the inhibition percentage:

% RSA = ((Abs Control- Abs Sample)/Abs Control) ×100

RSA = Radical Scavenging Activity
Abs Control = Absorbance of control
Abs Sample = Absorbance of sample

Table 1: List of sample for the Anti-oxidant activity.

Sample Code	Sample Name
Sample A	**Ethyl acetate extract of *Actinidia deliciosa***
Sample B	**Hydroalcholic extract of *Actinidia deliciosa***
Sample C	**Ethyl acetate extract of *Ocimum gratissimum***
Sample D	**Mixture of ethyl acetate extract of *Actinidia deliciosa and***

3. Result:

3.1. Phytochemical analysis :

The phytochemical analysis helpful for the determination of the some active biological components of the *Actinidia deliciosa* and *Ocimum gratissimum,* the result of the phytochemical test for alkaloids, flavonoid, phenols, amino acids, glycosides was prescribed in Table 2.

According to the Table 2 the n-hexane extract don't contains any phytochemicals, while the both the other extracts, i.e. ethyl acetate and hydroalcholic extract, of both the plants contains important phytochemicals like flavonoids, phenols which are important for the antioxidant activity of the plants.

Table 2: List of phytochemicals test performed for the determination of different phytochemicals in both the plant extract.

Test	Test Name	Plant name					
		Actinidia deliciosa			*Ocimum gratissimum*		
		Solvent					
		n-Hexane	Ethyl acetate	Hydro-alcoholic	n-Hexane	Ethyl acetate	Hydro-alcoholic

Alkaloids	**Dragendroff's test**	**x**	**x**	**x**	**x**	√	√
	Mayer's test	**x**	**x**	**x**	**x**	√	√
Flavonoid	**Lead Acetate test**	**x**	√	√	**x**	√	√
	Shinoda Test	**x**	**x**	√	**x**	√	√
Phenols	**Ferric chloride test**	**x**	√	√	**x**	√	√
Amino acids	**Ninhydrin test**	**x**	√	√	**x**	√	√
Glycosides	**Keller-Kiliani test**	**x**	√	√	**x**	**x**	**x**

3.2. TLC Analysis :

Using the mobile phase of the 1:1 combination of ethyl acetate and n-hexane, the TLC analysis of the ethyl acetate and hydroalcholic extracts of *Actinidia deliciosa* and *Ocimum gratissimum* leaves is carried out, and the Rf value of the various spots generated is stated in the Table 3 and 4 respectively.

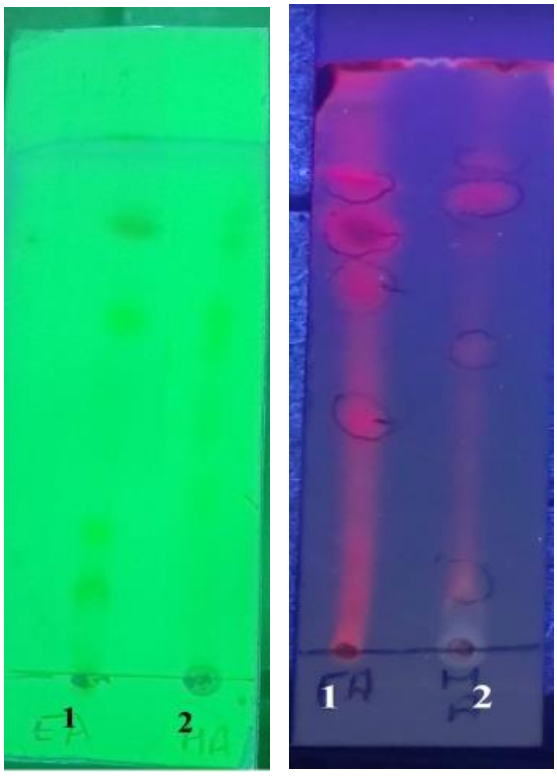
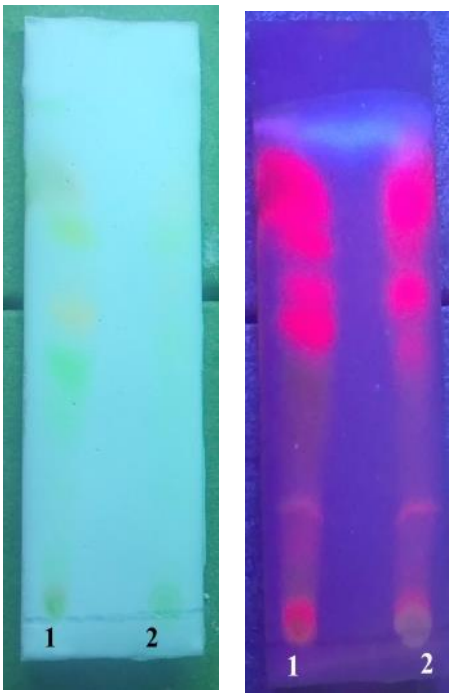


Table 3: Rf value of the extract of *Actinidia deliciosa*

Plant species	Extract	Distance travelled by compound(cm)	Distance travelled by solvent (cm)	R_f value
Actinidia deliciosa	Ethyl acetate (A)	2.5	6.0	0.416
		3.8	6.0	0.633
		4.5	6.0	0.750
		5.0	6.0	0.800
	Hydroalcholic	1.0	6.0	0.166
		3.0	6.0	0.500
		4.7	6.0	0.783
		5.2	6.0	0.866

A



A B

Table 4: Rf value of the extract of *Ocimum gratissimum*

Plant species	Extract	Distance travelled by compound(cm)	Distance travelled by solvent (cm)	R_f value
Ocimum gratissimum	Ethyl acetate	3.4	8	0.425
		5.6	8	0.700
		6.3	8	0.787

		7.9	**8**	**0.987**
	Hydroalcholic	**2.8**	**8**	**0.350**
		4.9	**8**	**0.612**
		6.8	**8**	**0.850**
		7.3	**8**	**0.912**

3.3. HPTLC Analysis :

The HPTLC system, was utilized to conduct High Performance Thin layer Chromatography analysis of the hydroalcoholic and ethyl acetate extracts of *Actinidia deliciosa*, and found absence of quercetine

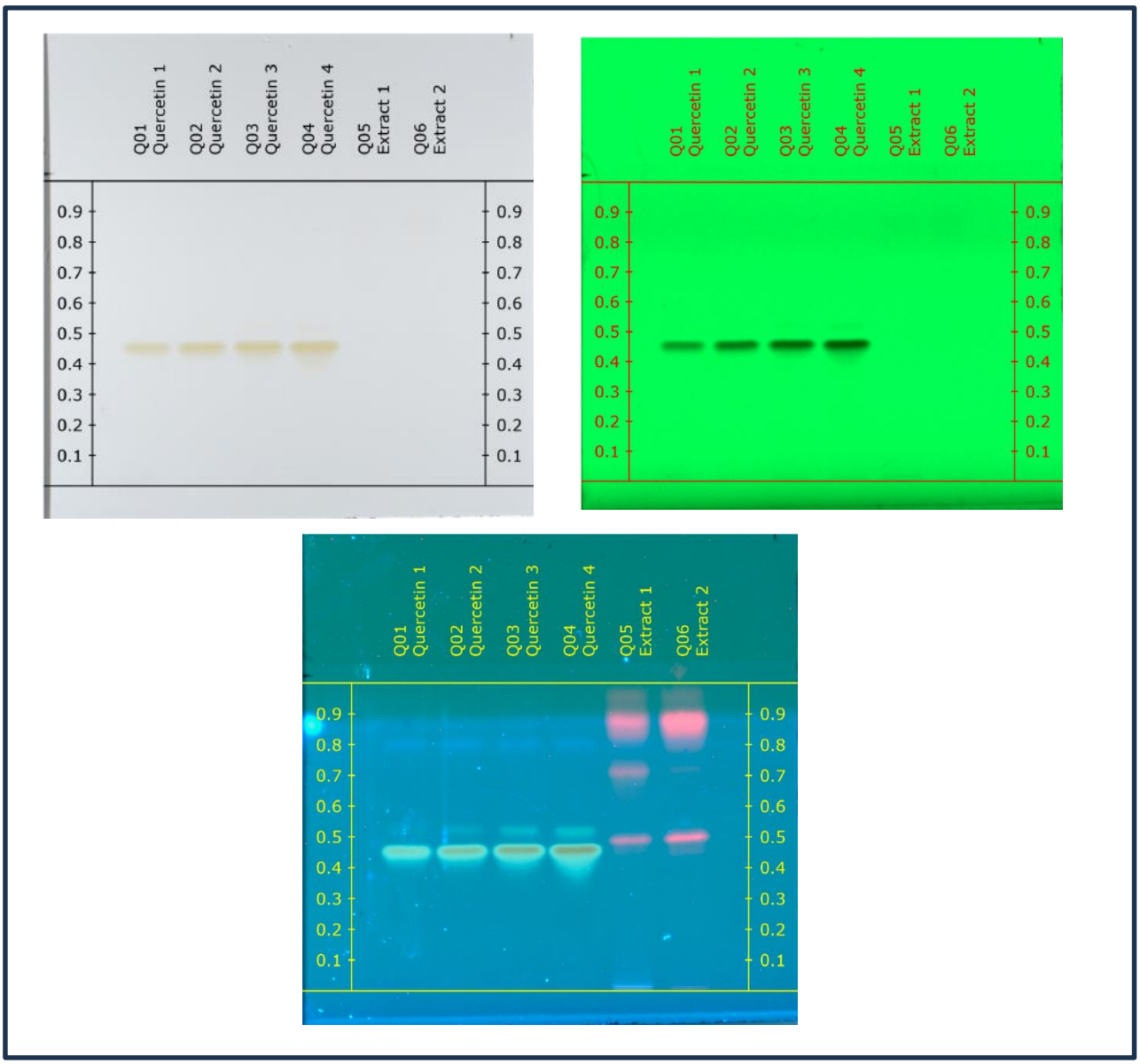


Fig 1 : HPTLC of hydroalcoholic and ethyl acetate extracts of *Actinidia deliciosa* leaves using Quercetin as marker

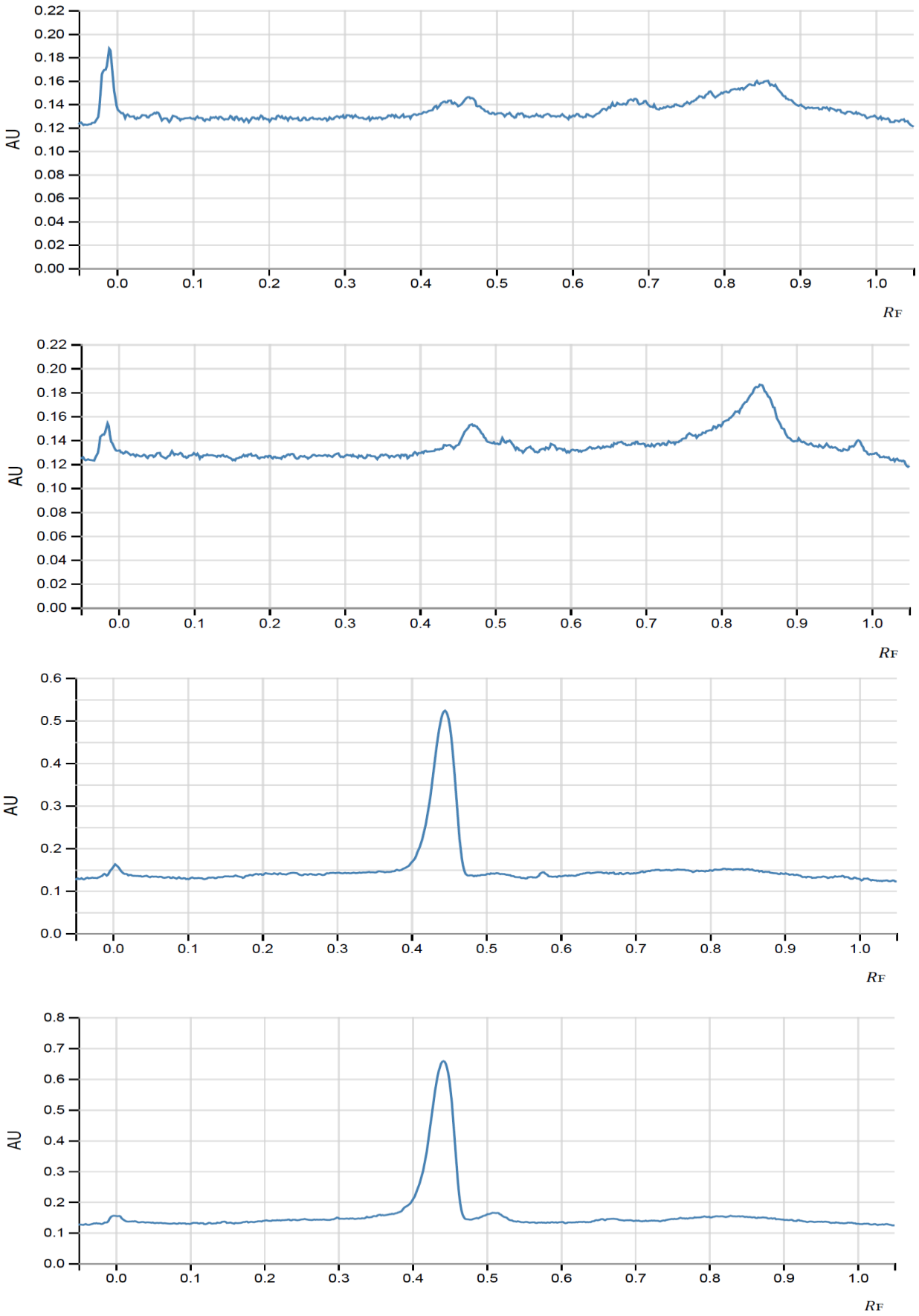
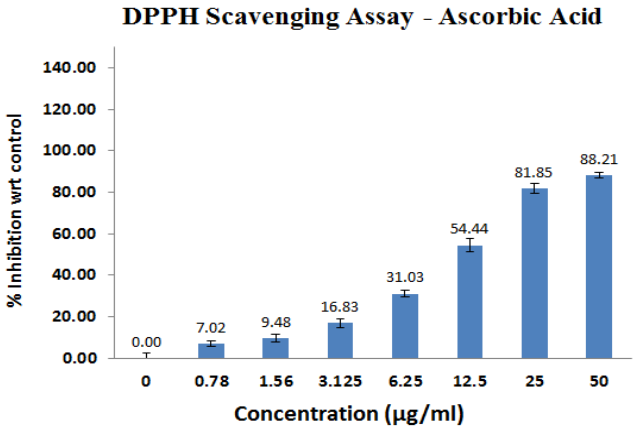
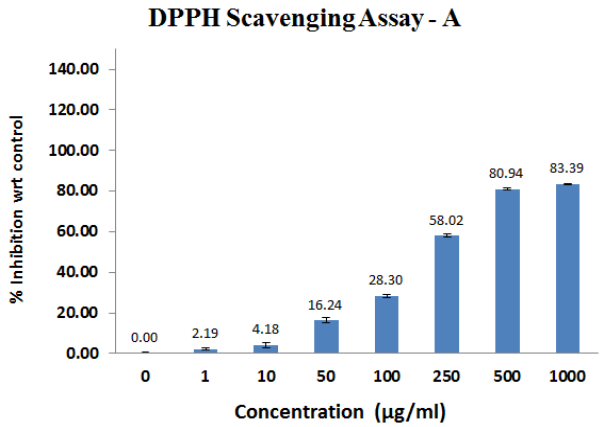


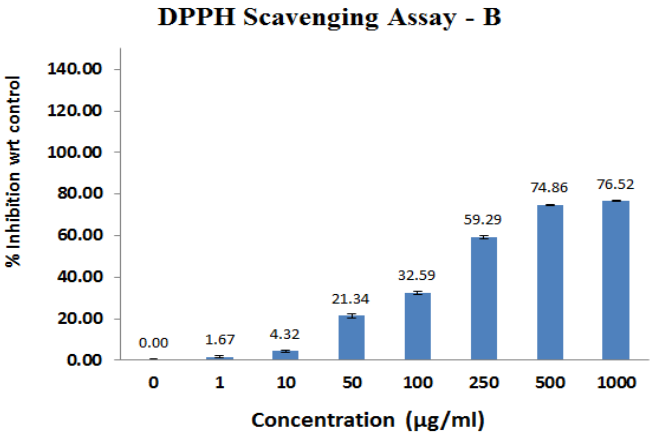
Fig 2 : HPTLC peaks at the wavelength of 354 nm

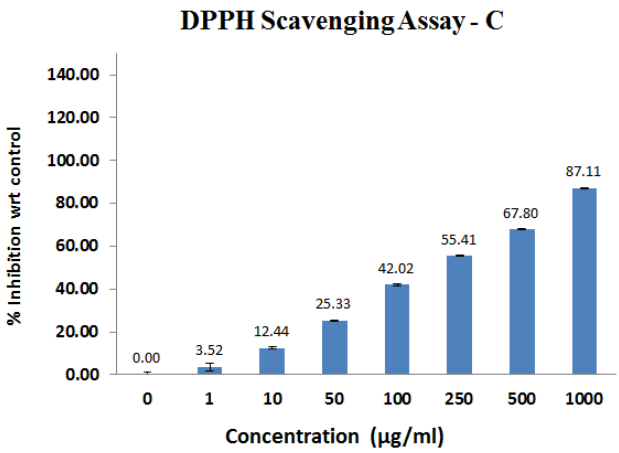
3.4. Anti- oxidant activity

Results obtained from the experimental work, Antioxidant activity (DPPH Assay) was estimated in samples and 50% inhibitory concentration (IC50) was mentioned in Table . Sample D was found to be more active among all the samples. 194 µg, 192.9 µg 166.2 µg and 312.2 µg of samples A, B, C and D were found more to 10.32 µg of standard Ascorbic acid.









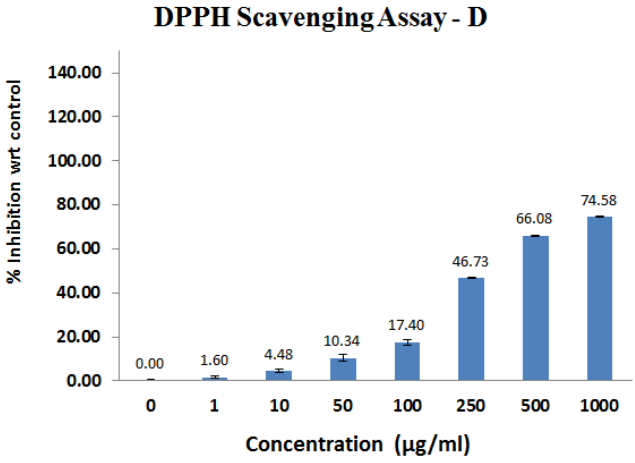


Table 5: *IC_{50}* value of the different sample for the Antioxidant activity

Sample code	IC_{50} value (µg/ml) (Mean ± SEM)
Ascorbic Acid	10.32 ± 0.0262 µg/ml
A	194 ± 0.0294 µg/ml
B	192.9 ± 0.0374 µg/ml
C	166.2 ± 0.0439 µg/ml
D	312.2 ± 0.0319 µg/ml

4. Conclusion:

Being a fruit plant the *Actinidia deliciosa* plant leaves is an agricultural west and the leaves of *Ocimum gratissimum* is been used as a medicinal plant from the decided and also contents the good antioxidant properties. But on the basis of above-mentioned result of the, antioxidant activity using DPPH scavenging model, the leaves of *Actinidia deliciosa* show better antioxidant activity as compared with the *Ocimum gratissimum leaves,* as it also contains beneficiary phytochemicals which can show better antioxidant activity and there is also the major increase in the antioxidant activity when we mix the both plant extract. Hance on the on the basis of the above result the leaves of *Actinidia deliciosa* instead of considering the agricultural west, it can also be used for its medicinal plant which can be helpful to fight against various disease which may cause due to oxidative stress, as the leaves contains antioxidant properties

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