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Quantitative Analysis of Gallic acid and Quercetin by HPTLC and *In vitro* Antioxidant activity of *Averrhoa carambola* Linn

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ABSTRACT

The preliminary phytochemical screening of ethanolic extracts of *Averrhoa carambola (A. carambola)* leaves were done using the standard protocol. The findings of phytochemical analysis exhibited the occurrence of Carbohydrates, Alkaloids, Steroids, Tannins, Vitamin C and flavonoids by using the Folin-Ciocalteu technique and the aluminum chloride colorimetric technique, respectively, the whole phenolic or flavonoid amounts were evaluated and were found 194.48±0.723 mg/g of dry extract as equivalent to gallic acid and 54.83±0.108 mg/g of dry extract as equivalent to quercetin respectively. *In vitro*, antioxidant activity was evaluated using 2,2-diphenlyl-1-picrylhydrazyl (DPPH), Hydrogen peroxide (H_2O_2), and Nitric oxide (NO) method. Ethanolic extract of *A. carambola* leaves showed good *In vitro* antioxidant action. Thin layer chromatography (TLC) of the ethanolic extract was carried out with gallic acid and quercetin as the reference biomarkers. HPTLC (High-Performance Thin Layer Chromatography technique was applied to detect spots and quantification for gallic acid and quercetin. The R_f values of gallic acid and quercetin were found 0.25 and 0.53 respectively. The amounts of gallic acid and quercetin were found to be 502.7 µg and 458.3 µg/100 mg of the ethanolic extract of *A. carambola* leaves separately.

Keyword: *A. carambola*, Phenolic, Flavonoid, Gallic acid, Quercetin, Antioxidant, TLC and HPTLC.

INTRODUCTION

Traditionally, plants are considered an essential source of medicines and playing a crucial role in the health of the overall community. In addition, biological importance of secondary metabolites of plants on humans has been known for a long time [1].

A. carambola commonly referred as star fruit or carambola, is a well-known plant that belongs to the family Oxalidaceae. *A. carambola* is mainly grown in southern China, Southeast Asia, India, as well as Northern South America. The leaves of A. carambola are normally used to treat coughing, headaches, chicken-pox, ringworm, vomiting, fevers, angina, aphthous stomatitis, diabetes, and hangovers[2,3,4].

According to numerous phytochemical and

pharmaceutical studies, the extract of *A. carambola* leaves is a reliable source of phenolic, flavonoids, saponins, alkaloids, and tannins, etc. The various reported bioactive compounds present in the leaves such as Vitamin C, quercetin and gallic acid are responsible for specific healing properties[5,6,7].

Plants rich in phenolic and flavonoids mixtures such as gallic acid and quercetin are primarily capable for health advantages. Plants those have high amount of phenolic and flavonoids content, produce more significant antioxidant activity. Antioxidants are essential in the management of inflammation, diabetes, cancer, dementia, and Alzheimer's disease[8,9].

In the study of phytoconstituents analysis, Thin-layer chromatography (TLC) and High-performance thin-layer chromatography (HPTLC) techniques has been employed frequently. These techniques helps in the qualitative and quantitative analysis of plants constituents in tiny amounts. The uses of these techniques for evaluation of herbal medicines has grown, as well as many herbal pharmacopoeias, now involves these techniques for the identification and standardization of phytoconstituents and phytoproducts. These techniques tells the quality and quantity of the phytoconstituents in plant medicine[10,11]. Structures of Quantified compounds (Gallic acid and Quercetin) are available in Figure 8.

This study aimed to perform the extraction, extractive value determination, preliminary phytochemical screening, entire phenolic and flavonoid contents determination, *In vitro* antioxidant activity determination by various methods, TLC and HPTLC for measurement of the two marker phytoconstituents gallic acid and quercetin in ethanolic extract of *A. carambola*.

MATERIAL AND METHODS

Collection and Authentication of Plant Materials

The fresh leaves of *A. carambola* L. were gathered from the garden of the Dariyapur Bujurg, Distt Amroha Uttar Pradesh, India. Plant materials were taxonomically detected and verified by Dr. Sunita Garg, Former Chief Scientist, and Head, of Raw Materials Herbarium and Museum, Delhi (RHMD), CSIR- NIScPR as *A. carambola* L (Family: Oxalidaceae) with Authentication No.-NIScPR/RHMD/Consult/2021/3914-15-1. Plant samples were submitted in the herbarium of the same laboratory.

Chemicals and Drugs

Glacial acetic acid, Toluene, Ethyl acetate, Ethanol, Methanol, Hydrogen peroxide, and Petroleum ether (60-80°C) were acquired from Central Drug House (CDH) New Delhi. DPPH and Ascorbic acid were obtained from Sigma Aldrich. Gallic acid and Quercetin were procured from Yucca Enterprises, Mumbai. All the chemicals and drugs used were of laboratory grade.

Leaves Extract Preparation

The leaves of *A. carambola* were collected and dried in shade at room temperature until the leaves became well-dried. After drying leaves crushed it into a coarse powder. 50 g of dried coarse leaf powder was taken in Soxhlet and extracted with 250 mL of Petroleum ether (60-80°C) for defatted and it was further extracted with Ethanol. The extract was dried using the water bath and preserved in a desiccator for further use. The extractive value of Ethanolic extract was measured[12,13].

Preliminary Phytochemicals Investigation

The preliminary phytochemical investigations of ethanolic extract of *A. carambola* leaves were performed to identify different types of phytoconstituents such as carbohydrates, Amino acids, Proteins, alkaloids, saponins, steroids, tannins, Vit C and Flavonoids[12,14].

Total Phenolic Contents Determination

The *Folin ciocalteu* (FC) technique was used for the estimation of whole phenolic amount of ethanolic extract of *A. carambola* leaves. 1 mL extract of 1 mg/mL conc. was added with 1 mL of FC chemical and after five minutes, ten ml of seven% sodium bicarbonate solution was mixed to the above mixture. After A few seconds, thirteen ml of distilled water was mixed systematically. The above solution was stored in the dark for ninety minutes at room temperature, after this the absorbance was noted at 760nm. The entire phenolic content was estimated from standard curve prepared by gallic acid. Total Phenolic Content are described as gallic acid equivalent (mg/g of dry extract)[15,16].

Total Flavonoid Contents Determination

The whole flavonoid amount of the ethanolic

extract of *A. carambola* leaves was determined by aluminum chloride spectrophotometer method. Firstly 1 mL extract of 1mg/mL was taken in a test tube and mixed with 2 mL CH_3OH, 0.1 mL aluminum chloride (10%), 0.1 mL potassium acetate, and 2.8 mL distilled water. The above mixture mixed thoroughly, stored for thirty min in dark and the absorbance was noted at 415nm using a UV-spectrophotometer. The standard quercetin solution was tested in the same way. The total flavonoid contents in *A. carambola* leaves extract are described as quercetin equivalent (mg/g of dry extract)[17].

In vitro Antioxidant Activity

DPPH radical scavenging assay

The Antioxidant activity of the ethanolic leaves extract of *A. carambola* at different concentrations against DPPH was tested. 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL of the leaves extract and ascorbic acid as reference in similar Concentration. as filled in test tube and in every test tube, DPPH solution was mixed in similar volume. After these two milliliters of methanol was mixed in each and every test tube and the test tubes were stored for ninety minutes in a dark room. After ninety minutes, all the test tube absorbance was noted at a wavelength of 517nm by the spectrometer. The inhibition percentages of the reference as well as the sample were calculated via equation 1. Inhibition Conc. 50(IC) value was calculated from the percentage inhibition vs conc. graph[18,19,20,21].

$$\text{Inhibition (\%)} = \frac{\text{Absorbance control - Absorbance Test}}{\text{Absorbance control}} \text{X} 100$$

All tests were performed in triplicate.

Hydrogen Peroxide (H_2O_2) Assay

Antioxidant activity by H_2O_2 was estimated according to the method of Ruch *et al.,* with slight modification[28]. H_2O_2 (40 mM) solution was made in phosphate buffer (50mM pH 7.4). 10, 20, 30, 40, 50, 60, 70, and 80 µg/mL conc. of the leaves extract and Ascorbic acid as a reference in similar conc. was filled in a test tube and mixed 2 mL of H_2O_2 into each and every test tube and after that added 2 mL of phosphate buffer solution (50mM pH 7.4) in each test tube. Absorbance was noted at 230nm by spectrophotometer. The inhibition percentages of the reference as well as sample were determined via above mention equation 1. IC_{50} value was calculated from the percentage inhibition vs conc. graph[22,23].

Nitric Oxide Scavenging Assay

The antioxidant activity of Ethanolic leaves extract of A. carambola at different conc was tested by the nitric oxide method. 10, 20, 30, 40, 50, 60, 70, and 80 µg/mL conc. of the plant leaves extract and ascorbic acid as a reference in similar conc. was filled in test tube and in every test tube 4 mL sodium nitroprusside (10 millimole), 1 mL phosphate buffer (7.4 pH and all test tubes was stored at room temperature for 150 minutes. After storage 0.5 mL mixture withdrawn from each test tubes and filled in new test tubes and added 1 mL sulphanilic acid and stand for few minutes. After that 1 mL of Napthyl Ethylene Diamine Dihydrochloride (NEDD) was mixed and again stored for thirty minutes. Absorbance of each test tubes noted at 540nm by spectrometer. IC_{50} value was determined from the percentage inhibition vs conc. graph[17,24].

Thin-layer chromatography (TLC)

The various solvent system was optimized for TLC analysis of Ethanolic leaves extract of *A. carambola* but finally, the below mentioned solvent system presented in Table 1 was used. The R_f value of different spot or solutes was determined by the below mention formula 1.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent front}}$$

Table 1: TLC and HPTLC solvent system for Ethanolic extract of *A. carambola*

Leaves	Solvent Mixture	Ratio	Detection
A. carambola	Toluene: Ethyl acetate: Glacial acetic acid	6:3:1	Day Light

Authors also wanted to find out how much amount of gallic acid and quercetin are present in ethanolic leaves extract of *A. carambola*. for this we used HPTLC technique because HPTLC is simple and cheaper technique as compared to other techniques.

Preparation of Standard Solution

Standard amounts of gallic acid and/or quercetin, each weighing 1 mg, were each mixed with methanol on their own to produce standard solutions with a concentration of 100 µg/mL.

Preparation of test solution

100 mg ethanolic extract of *A. carambola* was added in 10 mL ethanol and shaken for ten minutes and filtered. Filtrate was used for HPTLC analysis.

HPTLC Method Development

One of the most effective methods for determining the levels of phytoconstituents found in various plant parts is high-performance liquid chromatography (HPTLC). An aliquot of every dilution of the standard and test solutions (ethanolic extract) has been applied to a pre-coated TLC plate (5 by 10 centimeters with a 0.2mm aluminium base) by using Camag Linomat V. The aliquot was 10 microliters in volume. The chromatogram has been developed in a below mention solvent system mentioned in Table 1 in a saturated chamber. The plate that had been developed was first dried with a flow of hot air, then it was examined in a TLC scanner at an able to detect wavelength. The peak area, as well as the area under the curve, were plotted against the sample concentrations in order to generate a calibration curve. It was determined how much of the marker compound was present by employing an equation of regression based on the calibration curve[25,26].

Statistical analysis

The findings of study were indicated as the mean accompanied by the ± standard deviation (SD) of three separate attempts. From the regression plots, we were able to derive the IC_{50} values(the concentration at which there was a 50% reduction in activity). The findings were subjected to a one-way analysis of variance (ANOVA) wherever it was relevant to do so, and the significant difference ($P<0.05$) between the means was significant.

RESULTS

Extractive value

The Extractive value of the ethanolic extracts of *A. carambola* leaves is presented in Table 2.

Table 2: The result of the Extractive Value of the Ethanolic extracts of *A. carambola* leaves

S. No	Solvent	Extractive Value (%w/w)
1	Ethanol	7.308±0.46

Values are expressed as Mean±SD

Phytochemical analysis

The Phytochemical analysis of the ethanolic extract of A. carambola leaves appeared that it contains carbohydrates, alkaloids, saponins, steroids, tannins, Vitamin c and flavonoids. The finding of phytochemical screening is presented in Table 3. Such secondary metabolites are also recognized to have several pharmacological benefits.

Table 3: Results of phytochemicals screening of ethanolic extracts of *A. carambola* leaves

S. No	Name of the chemical test	Ethanolic extract
1	Test for Carbohydrate	
	Molish's test	+
2	Test for amino-acids	
	Ninhydrin test	-
3	Test for Proteins	
	Biuret test	-
	Million's test	-
4	Test for Alkaloids	
	Dragendroff's test	+
	Mayer's test	+
5	Test for Saponins	
	Foam test	+
6	Test for Steroid	
	Salkowski reaction	+
	Liebermann Burchard Reaction	+
7	Test for Tannins	
	Drug + 5% Ferric Chloride	+
	Drug+ Lead Acetate Solution	+
8	Test for vit C	+
9	Test for Flavonoids	
	Shinoda test	+
	Alkaline Reagent Test	+
	Zinc Hydrochloride Test	+

(+) = Present; (-) = Absent

Total Phenolic content

Using a UV-spectrophotometric technique, the overall phenolic material of the ethanolic leaves extract of *A. carambola* leaves was calculated. It was discovered that the total amount of phenolic content was 194.48±0.723 mg gallic acid equivalent/g weight of dry extract. The values shown are the mean±standard deviation of three distinct assessments.

Total flavonoids content

Using a UV-spectrophotometric technique, the flavonoid substance of the ethanolic leaves extract of *A. carambola* leaves was determined. It was discovered that the total flavonoid substance was 54.83±.108 mg quercetin equivalent/g weight of dry extract. The values provided are displayed as the mean±standard deviation of three distinct assessments.

Antioxidant activity of Ethanolic Leaves Extract DPPH radical scavenging assay

The DPPH test method uses a stable free radical called 2,2-diphenyl-1-picrylhydrazylhydrate, which can admit an electron to transform into a

stable moiety. *A. carambola* leaves extracts in ethanol demonstrated potent free radical scavenging abilities. The rising amount of the compounds affects the scavenging activity. The existence of antioxidants in the substances is indicated by the DPPH assay's transition from purple to yellow. Calculating *A. carambola's* % of scavenging activity and plotting it against conc. vs. % suppression Fig. 1. Various conc, notably 10, 20, 30, 40, 50, or 60, 70, as well as 80 g/mL, were used for the scavenging activity. The outcomes supported the discovery of a dose-dependent inhibition activity that displayed free radical scavenging activity. Strong antioxidant activity was demonstrated by *A. carambola's* ability to scavenge DPPH free radicals, with IC_{50} values of 87.±.252g/mL. The percent of the total DPPH scavenging actions of distinct concentrations of ascorbic acid and *A. carambola* leaves are presented in Table 4 and Fig. 1 and the IC_{50} value of *A. carambola* leaves and ascorbic acid is displayed in Table 5.

Table 4: Percentage of scavenging activity of Ascorbic acid and *A. carambola* leaves by DPPH method

S. No	Conc.(µg/mL)	(%) Inhibition (Mean±SD	
		Ascorbic acid	*A. carambola* leaves
1	10	27.13±0.024	11.94±0.064
2	20	32.19±0.016	23.58±0.09
3	30	38.26±0.048	30.67±0.057
4	40	44.23±0.024	35.43±0.062
5	50	51.42±0.009	39.98±0.012
6	60	56.48±0.065	46.86±0.016
7	70	62.04±0.032	50.30±0.048
8	80	68.12±0.097	55.67±0.062

Table 5: IC_{50} for Ascorbic acid and *A. carambola* during DPPH Method

S. No	Substance	IC_{50} (µg/mL)
1	Ascorbic Acid	41.73±0.098
2	A. carambola	68.11±0.252

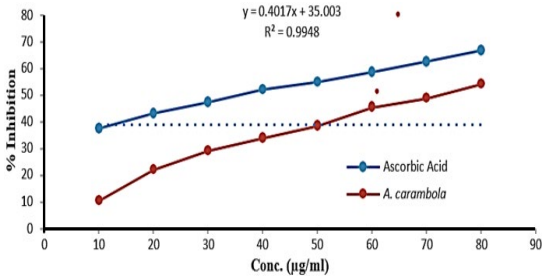

Fig. 1. Percentage inhibition of *A. carambola* as compred to ascorbic acid by the DPPH method

H_2O_2 Scavenging Assay

H_2O_2 is quickly disintegrated into oxygen and water and this may yield hydroxyl ions. Hydroxyl ions may start lipid peroxidation as well as disruption DNA in the body[27,28]. The percentage of H_2O_2 scavenging activities of different conc. of ascorbic acid and *A. carambola* are presented in Table 6 and Fig. 2. IC_{50} value of *A. carambola* leaves and Ascorbic acid is displayed in Table 7.

Table 6: Percentage of scavenging activity of Ascorbic acid (Vitamin C) and *A. carambola* during H_2O_2 Radical Scavenging Method

S. No	Conc. (µg/mL)	Inhibition (%) (Mean±SD)	
		Ascorbic acid	*A. carambola* leaves
1	10	10.76±0.056	10.16±0.012
2	20	22.43±0.024	20.93±0.046
3	30	33.40±0.064	29.68±0.036
4	40	44.47±0.098	41.65±0.076
5	50	55.03±0.042	50.30±0.024
6	60	67.51±0.086	60.06±0.092
7	70	88.63±0.012	68.81±0.03
8	80	90.24±0.08	70.93±0.018

Table 7: IC_{50} for Ascorbic acid and *A. carambola* during H_2O_2 radical scavenging method

S. No	Substance	IC_{50} (µg/mL)
1	Ascorbic Acid	45.93±0.172
2	A. carambola	62.64±0.942

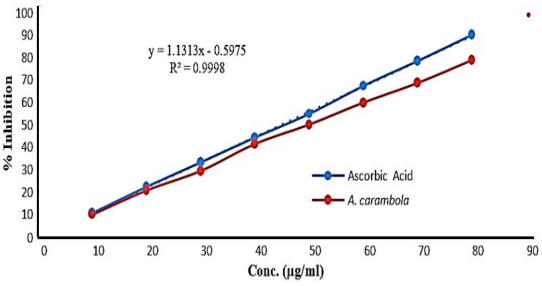

Fig. 2. Percentage inhibition of *A. carambola* as compared to Ascorbic axid by H_2O_2 radical scavenging method

Nitric Oxide Scavenging activity

The percentage (%) nitric oxide scavenging activities of distinct concentrations of ascorbic acid and *A. carambola* are presented in Table 8 and Fig. 3. leaves and Ascorbic acid is shown in Table 9.

Thin layer chromatography for qualitative analysis of Gallic acid and quercetin

TLC technique was mainly used for

qualitative analysis of extract. Findings of TLC analysis of the ethanolic extract of *A. carambola* showed the presence of Gallic acid and quercetin because R_f value of Reference Gallic acid and quercetin was similar as extract R_f value. The results of TLC are presented in Table 10 and Figure 4.

Table 8: Percentage of scavenging activity of Ascorbic acid (Vitamin C) and *A. carambola* during Nitric Oxide scavenging activity

S. No	Conc. (µg/mL)	Inhibition (%) (Mean±SD)	
		Ascorbic acid	*A. carambola* leaves
1	10	16.73±0.068	15.11±0.028
2	20	20.18±0.05	21.50±0.052
3	30	33.77±0.042	35.70±0.068
4	40	46.96±0.052	39.35±0.043
5	50	60.85±0.078	47.26±0.03
6	60	72.92±0.065	53.85±0.062
7	70	79.82±0.096	60.55±0.017
8	80	84.38±0.054	73.94±0.074

Table 9: IC_{50} for Ascorbic acid and *A. carambola* leaves during Nitric Oxide scavenging activity

S. No	Substance	IC_{50} (µg/mL)
1	Ascorbic Acid	42.41±0.098
2	A. carambola	66.84±0.782

Table 10: Number of spots and R_f values at wavelength UV254nm of Ethanolic extract of *A. carambola*

No of Spot	Standard R_f	*A. carambola* R_f
1	0.25 (Gallic Acid)	0.25 (Gallic Acid)
2	0.53 (Quercetin)	0.53 (Quercetin)
3		0.61 (Unknown)
4		0.65 (Unknown)
5		0.83 (Unknown)
6		0.91 (Unknown)
7		0.96 (Unknown)

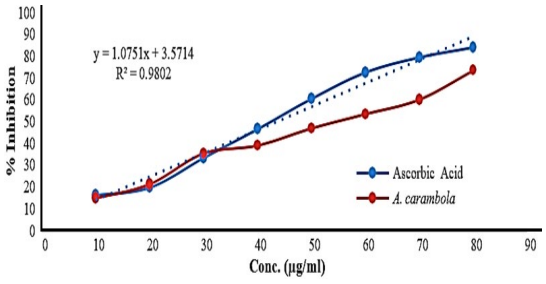

Fig. 3. Percentage inhibition of *A. carambola* as compared to Ascorbic acid by Nitric Oxide Scavenging activtiy

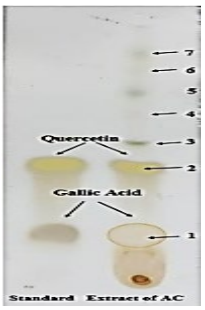

Fig. 4. Pictogram of developed TLC plate at daylight Standard=biomarker Gallic acid and quercetin, Extract of AC=ethanolic extract of *A.carambola* mobile phase, Toluene: Ethyl acetate: Glacial acetic acid (6:3:1, v/v)

HPTLC Analysis for Quantitative analysis of gallic acid and Quercetin

The HPTLC fingerprint and chromatogram showed the appearance of gallic acid and quercetin in the ethanolic leaves extract of *A. carambola*. (Table 11 and Fig. 5 Fig. 6, and Fig. 7). R_f value of gallic acid and quercetin of ethanolic leaves extract of *A. carambola* was identical to the standard at 0.25 and 0.53 when the plates were scanned at 254nm. The correlation coefficient was 0.995. The amount of gallic acid and quercetin in the ethanolic leaves extract of *A. carambola* was 502.7 µg and 458.3 µg/100 mg, respectively.

Table 11: Number of spots and R_f values at wavelength UV254 nm of Ethanolic extract of *A. carambola* leaves

No of Spot	Standard R_f	*A. carambola* R_f
1	0.25 (Gallic Acid)	0.05 (Unknown)
2	0.53 (Quercetin)	0.08 (Unknown)
3		0.09 (Unknown)
4		0.25 (Gallic Acid)
5		0.29 (Unknown)
6		0.30 (Unknown)
7		0.53 (Quercetin)
8		0.54 (Unknown)
9		0.83 (Unknown)
10		0.91 (Unknown)
11		0.96 (Unknown)

Fig. 5. HPTLC fingerprint of ethanolic extract of *A. carambola* scanned at 254nm

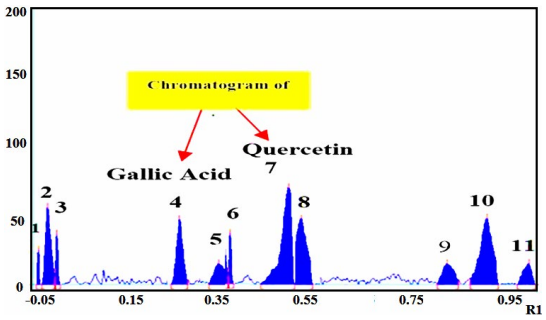

Fig. 6. Chromatogram of ethanolic extract of *A. carambola* leaves scanned at 254nm

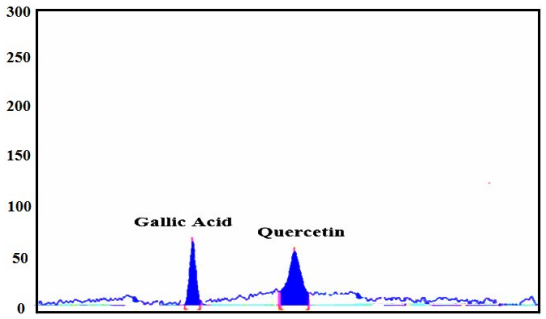

Fig. 7. Chromatogram of standard Gallic acid and quercentin scanned at 254nm

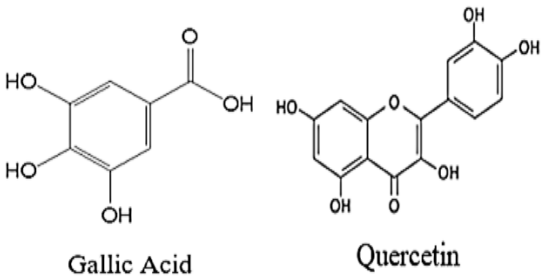

Fig. 8. Structure of Gallic acid and quercetin found in the ethanolic leaves extract of *A. carambola*

DISCUSSION

Extractive values play a very important role in the determination of quality and quantity of herbal drugs, also useful to evaluate the nature of phytoconstituents available in the plant drug and also useful in the assessment of particular phytoconstituents soluble in specific solvents[29]. Phytochemical analysis was done and the results showed the attendence of carbohydrates, alkaloids, saponins, steroids, tannins, Vitamin C, and flavonoids in ethanolic extracts of leaves. The findings of phytochemical analysis suggest that the ethanolic extracts of *A. carambola* leaves probably contain active phytoconstituents providing the basis for their use as a management for numerous illness[30,31].

Phenols and flavonoids are present in plants produce antioxidants activity. Hence, we could conclude that the phenols and flavonoids are accountable for the detected antioxidant activity in this research work. Phenols and Flavonoids are the greatest significant and miscellaneous group of phytoconstituents which are mainly distributed in higher plants with extraordinary therapeutic potential. Phenols and flavonoids have a beneficial action in the management and prevention of neurological disorders such as memory loss and can delay the process of neurological disorders. Phenol and Flavonoids compounds have crucial role in delaying the progression of Alzheimer's Disease [AD]. Many Studies recommended that phelonic and flavonoids have the ability to pass blood-brain barrier (BBB), which is important for management and prevention of neurological disorder such as memory loss. however, various flavonoid subcategories differ in ability to pass the BBB. In the management and treatment of memory loss and AD, flavonoids efficiency is attributed to the reduction of amyloid beta (A) toxicity and decreasing oxidative stress. Numerous phenolic and flavonoids such as gallic acid, rutin, catechins, quercetin, kaempferol, myricetin, and apigenin are useful in prevention and management of neurodegenerative diseases, cancer, inflammation etc. have been reported.[32]

The antioxidant activity of ethanolic leaves extract of *A. carambola* was measured by DPPH, H_2O_2, and NO methods. The explanation of each method are given below one by one. DPPH is extensively used to find out antioxidant activity of plant extract. It is simple and cheaper technique. DPPH dark in colour and crystalline in nature. It is prepared by free-radical elements that are stable. It is well known and a popular antioxidant method.[33]. DPPH radicals were scavenged by *A. carambola* leaves in a conc. dependent manner. Using DPPH, we found in this study *A. carambola* leaves have antioxidant activity. Similarly, the ethanolic leaf extract exhibited remarkable scavenging activity when compared to standard ascorbic acid by H_2O_2 and NO scavenging assay method. The ethanolic leaf extracts strongly scavenge in dose-dependent manner shown in Fig. 2 and 3.

Because of the free radical scavenging activity of *A. carambola* leaves, it can be useful in the management and prevention of numerous health problems causes by free radicals.

Free radicals such as nitric oxide, and hydrogen peroxide are well-recognized inducers of cell and tissue pathogenesis which may cause numerous human diseases such as neurodegenerative diseases, aging diseases, and as well as inflammatory diseases[25]. Antioxidants are extremely found in phytoconstituents having the Potential to secure the human body from injury caused by free radical caused oxidative stress. The antioxidant capacity of *A. carambola* leaves extract examined and significant results were found[20].

The HPTLC technique is a simple, specific, precise, sensitive, and accurate technique for the quantification of phytoconstituents from plant extract. We used this technique for the quantification of gallic acid and quercetin. This technique can be efficiently used for routine analysis of phytoconstituents as well as formulations containing any compounds[34]. The HPTLC analysis provided the amount of gallic acid and quercetin as 502.7 µg/100 mg and 458.3 µg/100 mg of ethanolic extract of *A. carambola* leaves respectively. Many Studies suggested that gallic acid and Quercetin are beneficial in the management and prevention of various diseases such as neurodegenerative diseases, cancer disease, inflammatory diseases, etc. Data collected from a variety of sources suggested that Gallic acid and Quercetin have the ability to reverse amnesia in rodents induced by scopolamine. Because it inhibits oxidative stress and also decreases acetylcholinesterase levels in the rodent brain. Gallic acid and Quercetin are commonly found in edible plants[32,35,36,37].

CONCLUSION

These unreported parameters may be helpful in establishing the diagnostic characteristics for the recognition of the *A. carambola* plant also the creation of a monograph on it.

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Conflict of interest

The author declare that we have no conflict of interest.

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