



Research Article

## Antimicrobial activity, Phytochemical screening by HPTLC, FTIR and GC-MS of the extracts of *Cissus quadrangularis* (L.)

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### Abstract

Finding new antimicrobial compounds or extracts is essential to combat the serious problem of rising bacterial resistance to traditional antibiotics. Plant chemicals' biodiversity is a valuable potential resource. The goal of this work is to identify the *Cissus quadrangularis* active ingredients that have antibacterial properties. Quercetin ( $R_f$ -0.6), myricetin ( $R_f$ -0.49), apigenin ( $R_f$ -0.68), gallic acid ( $R_f$ -0.35) are present in *C. quadrangularis*, which gives it powerful antioxidant properties. The plant extract contains  $34.8 \pm 0.014$  mg/g of total flavonoids, which is equal to quercetin, and  $20.46 \pm 0.01$  mg/g of total phenolics, which is equal to gallic acid. It was found that the extract has antibacterial activity against *E. coli*-ATCC 10231, *S. typhi* NCTC-786, *S. aureus*-ATCC-9144 and *B. subtilis*-ATCC 9372. This activity was revealed using the zone of inhibition. On the other hand, GC-MS analysis was used to identify more active chemicals. Phytoconstituents like n-Hexadecanoic acid, Phytol, Z-7-Tetradecenal, Campesterol, Gamma sitosterol, Tetracosanoic acid methyl ester, Squalene, Tocopherol, and Vitamin E. Gamma sitosterol, and Campesterol created several hydrogen bonds with the three targets protein (1AJ6, 7D6G & IGG4), according to an analysis of the docking results. Furthermore, the MM-GBSA energy analysis results showed that gamma sitosterol had a substantially higher binding affinity than folate to the active site of 7D6G. Together, these results suggest that campesterol and gamma sitosterol work synergistically, the antimicrobial properties of the *Cissus quadrangularis* extract are probably similar to ampicillin.

**Key word:** Anti-microbial activity, *Cissus quadrangularis*, GC-MS analysis, HPTLC, IR

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### Introduction

World Health Organization denotes that most of the developing countries rely on the herbal medicines to treat different disease. Demand of traditional medicines has been increasing day by day. Perennial plant, *Cissus quadrangularis* with tendril climber, a popular Indian traditional medicine, belongs to the family Vitaceae with the presence of numerous

bioactive compounds like alkaloids, tannins, flavonoids, phenolic compounds etc. which are effective against several degenerative diseases (Syed et. al., 2021). The plant consists quadrangular shape like stem, small leaves, and roots. According to Ayurveda, Homeopathy and Ethno-botany traditional system of medicines, roots of *C. quadrangularis* are generally used as healing element for bone fractures and

swelling (Rekha et. al. 2018). This climbing shrub is normally cultivated in the tropical regions of Africa, Sri Lanka, Malaysia, India etc. The plant is prominently mentioned in all ancient Ayurvedic scriptures and is used in traditional Indian folklore medicine to heal bone fractures. Ancient texts by Bhava Prakash and Chakra Dutta prescribe it as a general tonic, particularly for patients with fractures. Since then, it has been extensively used by bone setters for both external application and as an internal medicine taken with milk. The stem of *Cissus quadrangularis* is renowned in Ayurveda for its properties as an alternative, anthelmintic, dyspeptic, digestive, and tonic (Tiwari, Gupta, & Sharma, 2018). It is also used for treating eye and ear diseases, irregular menstruation, asthma, and complaints related to the back and spine.

The archeo-botanical revelation has proven the usage of this types of medicinal plants cultivated in middle gangetic region by the traditional healers since 2<sup>nd</sup> millennium BC (Chenniappan et. al. 2020). Apart from healing ailment, the stem of *C. quadrangularis* is also used in the treatment of constipation, gastric ulcers, conjunctivitis etc (Ayyanar et. al., 2008). The leaf and young shoots are used in many ailments like indigestion, haemorrhoid etc. As per Ayurveda system of medicine, it is further used in syphilis, piles, gout, leucorrhoea and other venereal diseases whereas in siddha system of medicine, it is used in the treatment of diarrhoea, dysentery, cuts, wounds, swellings and stomach ache etc (Chaudhari et. al., 2013). Panthong et al., (2006) revealed that the ethanolic extract of *C. quadrangularis* contained luteolin,  $\beta$ -sitosterol along with other bioactive flavonoids which can be useful as alternative medicine against numerous chronic disorders.

Oxidative stress is one of the main pathophysiological conditions of any degenerative diseases (Ayyanar et. al., 2008). It is already known that animal cells are not able to produce adequate amount of antioxidants like plant cells. Intake of synthetic or semi-synthetic antioxidants can lead to toxicity, carcinogenicity and mutagenicity. Natural antioxidants can mitigate and protect the cells from free radical damage without producing any adverse effect (Mansoori et. al. 2020). On the other side, in the time of worldwide growing infectious diseases, the count of deaths has increased. According to WHO, one of the main causes of morbidity and mortality are due to bacterial resistance against clinically available antibiotics. Bacterial resistance is one of the most discussed and unresolved topics in today's world. Here in the in-silico study the target protein 1AJ6 has been used which is basically novobiocin-resistant mutant of the n-terminal 24 kDa fragment of DNA gyrase B. So, in this target protein mainly, the antibiotic binds to novobiocin-resistant mutant of the n-terminal. Here the binding of campesterol and gamma sitosterol is comparable with the inbound ligand. Thus, it can be said that campesterol and gamma sitosterol can also be effective against antibiotic resistant bacterial strain of *E.coli* in in-vitro study (Rahimi et. al., 2016).

Additionally, the cost of treatment using modern antibiotics are pretty much expensive. So, there is obvious need of exploration of new antimicrobial agents (Parimala et. al. 2014). In search of major and lead secondary metabolites, phytochemical screening of *C. quadrangularis* through different high throughput analytical techniques are obvious

requirement to exhibit efficient activity against different diseases.

The present research has the objectives to explore the presence of numerous secondary metabolites in the extract by different analytical techniques using GC-MS, FTIR and HPTLC along with analysing antioxidant and antimicrobial potential of the stem part of this perennial plant, *C. quadrangularis* L.

## **Materials and Methods**

### **Plant material**

The stem part of *C. quadrangularis* was collected from the East Medinipur, West Bengal, India. The plant was identified and authenticated from Botanical survey of India, Kolkata. The voucher specimen number is CNH/Tech.II/2022/ 42-JIS/S-02.

### **Preparation of extract**

The muscular stems were washed (tap water followed by distilled water), cut into small pieces, shade dried in room temperature. Collected stems were pulverized until it became delicate powder by mechanical grinder. The powder (4.5 kg) was extracted in ethanol (25 lit) by maceration process. The extracted material was filtered and dried by using rotary vacuum evaporator (40-45 °C). The dried extract was stored at 4 °C till further use.

### **Proximate Analyses**

Ash value, moisture content, protein and fibre contain are variables selected for proximate analyse was performed by following previously published work of Chatterjee et al. 2019.

### **Phytochemical screening**

#### **Phytochemical screening: qualitative chemical test**

Chemical group test of the plant *C. quadrangularis* L. was carried out by using standard protocol to identify the presence of Alkaloids, Glycosides, Tannins, Tri-terpenoids, Steroid (Monteiro et. al. 2022).

### **HPTLC analysis**

Phytochemical profiling was carried out according to previously published work using HPTLC (High-Performance Thin-Layer Chromatography) with silica gel 60 F254 (Merck, Germany) on a pre-coated TLC plate (10×10 cm; 0.2 mm thickness).

#### **Phytochemical screening: Chemical derivatization**

Samples were run in plates with mobile phase Chloroform: Ethyl acetoacetate: Formic Acid at a ratio 5:4:1v/v/v. Post-chromatogram microchemical derivatization of developed plates was carried out using a CAMAG Derivatizer comprised of automated spraying nozzles, which subtly scatter droplets of derivatizing reagent onto HPTLC plates. A 2% w/v  $\text{FeCl}_3$  solution was dissolved in ethanol, and the solvent mixture was neutralized by 5%, a few mL dil. NaOH until the precipitate was formed. The Ferric hydroxide precipitate was filtered out, and the filtrate was collected and stored in an amber glass container. Alcoholic  $\text{FeCl}_3$  solution, p-anisaldehyde, and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) solution were pushed into nozzles of the derivatizer, which delivered the reagent on the developed HPTLC plate for visualizing

polyphenols, terpenes, phytosterols and oxidizing agents respectively. Each derivatizing plate was placed into TLC scanner 4 for documentation, and images were captured at 254nm (fluorescence), 366nm, and white light (416nm) (Ashraf et. al. 2021).

#### ATR-FTIR

Visible bands of derivatized plates were scrapped and dissolved in acetone. Further, silica was filtered, and filtrate was dried. With just minor modifications from Chatterjee et al., 2021, ethanolic extract of *C. quadrangularis*, and final filtrate were deposited on the ATR-FTIR, and the spectrum data was acquired using an Alpha II FTIR (Bruker, Germany) and were compared with standards. Prior to each measurement, the background spectrum of air was examined.

#### Phytochemical screening: HPTLC fingerprinting

A quantitative analysis of ethanolic extract of *C. quadrangularis* was subjected to HPTLC (Muttentz, Switzerland) operated by Vision Cat software analysis. The sample solution (1mg/mL) was centrifuged for 5 min and the supernatant liquid was collected. Similarly stock solutions of different phenolic and flavonoids (1mg/mL) standards were also prepared at same concentration. The sample solution and standard solutions were loaded on precoated plate (20cm \* 10cm) of TLC silica gel 60 F<sub>254</sub> using Linomat 5 applicator (CAMAG, Muttentz Switzerland) with a 10µl Hamilton syringe. A CAMAG Twin trough chamber was presaturated for 15min with mobile phase Chloroform: Ethyl acetoacetate: Formic Acid at a ratio 5:4:1v/v. Separation of the polyphenolic group was done with a migration distance up to 8cm. The sample loaded plates are allowed to dry and the chromatograms were placed in a UV chamber to visualize those spots at shorter (254nm) and longer wavelength (366nm) along with photo documentation (Ashraf et. al. 2021).

#### Phytochemical screening: GC-MS Analysis

The ethanolic extract of *C. quadrangularis* was subjected to GC-MS analysis. The GC-MS was done from Central University of Punjab using instrument Shimadzu QP 2010 Ultra GC MS. The carrier gas used as Helium with at flow of 1.0 ml/min. The identification of components was based on NIST libraries comparing their retention time (Chatterjee et. al. 2022).

#### In vitro antioxidant assay

##### Total phenolic content

The hydroxyl Group of the phenolic compound in the plant is responsible for the antioxidant properties due to its neutralizing or scavenging properties. Extract, Gallic acid (standard), Folin-Ciocalteu reagent and sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) are used to measure the total phenolic content (TPC). The TPC was measured at 765nm using some modification of Chatterjee et. al. 2019). The result obtained from the gallic acid standard curve and expressed as gallic acid equivalents in milligrams per gram of dry extract.

##### Total flavonoid content

Flavonoid content of the following extract was determined by slide modification of Rabrta et al (Olivia et. al. 2021).

#### DPPH Scavenging Activity

The DPPH assay was carried out to measure antioxidant activity of the extract *C. quadrangularis*. The assay was carried out by slide reform of Chatterjee et. al. 2019 & Nurul et. al. 2018 at 517nm. The antioxidant activity was determined by this equation:

$$\% \text{ Scavenging activity} = (\text{Abs of control} - \text{Abs of sample}) / \text{Abs of control}$$

#### Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) radical scavenging activity

The Ruch et al. (1989) method was applied to quantify *C. quadrangularis*'s H<sub>2</sub>O<sub>2</sub> scavenging activity. Various quantities of a *C. quadrangularis* solution were combined with a 40 mM H<sub>2</sub>O<sub>2</sub> solution. After ten minutes, absorbance was measured at 230 nm. Ascorbic acid was handled as reference antioxidant, while phosphate buffer served as the blank.

$$\text{H}_2\text{O}_2 \text{ radical Scavenging activity} = (\text{A}_{\text{Control}} - \text{A}_{\text{test}}) / \text{A}_{\text{Control}} * 100$$

Where, A<sub>Control</sub>: The absorbance of the control reaction

A<sub>test</sub>: The absorbance in the presence of the sample of *C. quadrangularis*

#### Superoxide anion scavenging activity

The superoxide anion scavenging activity of *C. quadrangularis* was measured using a slightly modified version of the methodology of Liu et al. and Ye et al. Nicotinamide adenine dinucleotide (NADH) solution (468 mmol/L) and nitroblue tetrazolium (NBT) solution (156 mmol/L NBT) were combined with the sample (*C. quadrangularis*). The reaction was initiated by mixing the aforementioned solution with 100 ml of phenazine methosulfate solution (60 mmol/L). At 560 nm, the absorbance was measured after five minutes of incubation at 25°C. A decrease in absorbance within the reaction mixture signified a rise in the activity of superoxide anion scavenging. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ inhibition} = (\text{A}_{\text{Control}} - \text{A}_{\text{test}}) / \text{A}_{\text{Control}} * 100$$

Where, A<sub>Control</sub>: The absorbance of the control reaction

A<sub>test</sub>: The absorbance in the presence of the sample of *C. quadrangularis*

#### ABTS scavenging activity

ABTS scavenging activity was performed by the slide modification of Chatterjee et al, 2019 using ABTS salt, potassium persulfate at 734 nm. After comparing *C. quadrangularis*' ability to scavenge ABTS with ascorbic acid, the % inhibition was computed as follows:

$$\text{ABTS radical scavenging activity (\%)} = (\text{A}_{\text{Control}} - \text{A}_{\text{test}}) / \text{A}_{\text{Control}} * 100$$

Where, A<sub>Control</sub>: The absorbance of the control reaction

A<sub>test</sub>: The absorbance in the presence of the sample of *C. quadrangularis*

#### Determination of antibacterial activity of crude extracts *C. quadrangularis*.

##### Bacterial Strains.

The extract *C. quadrangularis* was tested against gram-negative bacteria, *Salmonella typhi* (NCTC-786) and *E. coli* (ATCC 10231) and gram-positive bacteria, *Bacillus subtilis*

*Antimicrobial activity, Phytochemical screening by HPTLC, FTIR and GC-MS of the extracts of Cissus quadrangularis (L.)* (ATCC 9372) and *Staphylococcus aureus* (ATCC-9144). The bacterial strains were gifted from Central Drug Laboratory, Kolkata.

### Preparation of Disc for antibacterial activities

The antibacterial activity of extracts on test bacteria was examined by disk diffusion method by Ebtihal Alsadig Ahmed et al. 2020 (Mohamed, Muddathir & Osman, 2020). Different extract concentration (50, 100, 150, 200 & 500 mg/mL) was filtered through 0.45 mm membrane filter (Millipore, Bedford, MA). The sterile paper disc (Whatman #2paper, 6 mm in diameter) was soaked in the different extract, and the disks left to dry and remove the excess of solvents. These disks were used for antibacterial activity assessment.

### Disc Diffusion Method

One full loop of bacteria was spread over the surface of Mueller Hinton Agar (MHA) plate and allowed half an hour. Then the prepared antibacterial disc was placed over the lawn and pressed slightly along with positive and negative controls. The inoculated plates were incubated for 24 h at 37°C. After incubation, the diameter of the inhibition zone was measured. Discs (6 mm diameter) of Ampicillin (30 µg/ml disc) was used as positive control. Ampicillin which has been used as a broad-spectrum antibiotic. Each experiment was run three times, and the average of the outcomes was determined (Mohamed, Muddathir & Osman, 2020).

### Compound and receptor preparation

The structures of the three compounds, namely, ampicillin, campesterol and gamma sitosterol were retrieved from PubChem in SDF format (Kim et al, 2023). The structures were subsequently prepared using the LigPrep module in Schrödinger, with the OPLS4 force field (Lu et al, 2021). All possible ionisation states of the compounds at pH 7.0 ± 2.0 were generated with Epik (Shelley et al 2007). The original ionisation states were also included. The chiralities were preserved and a maximum of 32 structures were generated per ligand.

The PDB structures 1AJ6 (topoisomerase), 1GG4 (hydrolase inhibitor), and 7D6G (oxidoreductase) were retrieved from the Protein Data Bank (PDB) (Berman et al, 2000; Dealwis et al. 1995), and prepared using the Protein Preparation Wizard module in Schrödinger. Any missing side chains and loops were filled in with Prime, and the optimal ionisation states of Gln, Asn, His, and hydroxyl groups at pH 7.0 were calculated with PROPKA (Jacobson et al, 2004; Olsson et al, 2011). All water molecules at a distance greater than 3.0 Å from the hetero groups were removed, and the structures were finally

subjected to restricted minimisation using the OPLS4 force field.

### Grid generation and docking

For ligand docking to the PDB structure 1AJ6, a cubic grid was prepared around the binding site of the bound inhibitor, novobiocin. The x, y, and z coordinates of the centre of the grid were 60.094, -7.037, and 34.752, respectively, and the dimension of the grid was 30 Å on all sides. The grid parameters were validated by redocking the bound inhibitor (novobiocin) using Glide XP (Friesner et al, 2006; Halgren et al 2004). The RMSD values between the crystal and docked poses were determined for assessing whether the native binding pose could be predicted using the selected grid and docking parameters. The grid for 7D6G was prepared around the binding site of folate. The coordinates of the centre of the grid were 33.062, 42.488, and 7.887, and the dimensions of the cubic grid were 30 Å on all sides. The grid parameters were similarly validated by redocking the bound folate to the binding site, and the RMSD between the docked pose and crystal pose was determined.

Due to the absence of bound inhibitors, the grid for 1GG4 was prepared around the druggable site that mapped to the substrate binding site of the protein. The top five druggable sites of 1GG4 were initially predicted using SiteMap, following which the predicted druggable sites were mapped to the substrate binding residues reported in UniProtKB (Bateman et al. 2023; Halgren, 2007) The x, y, and z coordinates of the centre of the grid for 1GG4 were 5.613, 75.226, and 39.613 respectively. The dimension of the cubic grid for 1GG4 was 3.37 Å on all sides. The three prepared compounds were subsequently docked to the three targets using Glide XP. The molecular mechanics with generalised Born and surface area solvation (MM-GBSA) energy of the three compounds was additionally determined using the OPLS4 force field, with the VSGB solvation model (Lu et al, 2021).

### Statistical analysis

Results of the study were based on analysis of variance (ANOVA) and Differences were considered significant at  $p < 0.05$ .

### Result

#### Proximate Analyses

Table 1 displays the findings of the plant's proximate analysis of *C. quadrangularis*. It demonstrates that the plant has carbohydrate (53.45±0.15 %), protein (3.77±0.013 %), ash (2.89±0.01 %), and fibre (15.49±0.02 %).

**Table 1: Proximate composition of mature plant *C. quadrangularis***

| Sl no | Components of Proximate Analyse | %          |
|-------|---------------------------------|------------|
| 1     | Carbohydrate                    | 53.45±0.15 |
| 2     | Protein                         | 3.77±0.013 |
| 3     | Ash                             | 2.89±0.01  |
| 4     | Fibre                           | 15.49±0.02 |

### Phytochemical screening: qualitative chemical test

The preliminary phytochemical study of the extract of *C. quadrangularis* revealed the presence or absence of various

secondary metabolite in Table 2. Standard protocol was followed which primarily expressed the

presence of reducing sugar, cardiac glycoside, tri-terpenoid, flavonoid, phenolic acid (Peruma et. al. 2012). The present

study is further subjected to HPTLC analysis and free radical scavenging activity.

**Table 2. Preliminary phytochemical screening of the extract of *C. quadrangularis***

| Sl No. | Test for Phyto-constituents | Extract |
|--------|-----------------------------|---------|
| 1      | Glycosides                  | -       |
| 2      | Reducing sugar              | +       |
| 3      | Cardiac Glycosides          | +       |
| 4      | Steroid Test                | -       |
| 5      | Tannin Test                 | -       |
| 6      | Triterpenoids               | +       |
| 7      | Flavonoid                   | +       |
| 8      | Alkaloid                    | +       |
| 9      | Saponin Test                | -       |
| 10     | Phenolic Acid               | +       |

The number of soluble materials present in the extract is determined by water extractive and alcohol extractive values. Based on this value, a suitable solvent is chosen to get the highest quantity of phytochemical constituents (Kannan et. al., 2019). The ethanolic extract of *C. quadrangularis* showed the presence of several polyphenolic, flavonoid, and tri-terpenoid compounds by the phytochemical analysis, which led to further antioxidant study. Nowadays, the identification and isolation of these polyphenolic groups have become more crucial because of their various therapeutical activity. The polyphenolic group helps to balance the free radical in body due to their radical scavenging activity and reverse some of the damages caused by free radicals (Djeridane et. al., 2019). In addition, the extract which exhibit higher free radical scavenging activity and antioxidant activity show higher value of TPC, TFC and exercised a good correlation between these parameters (Parejoa et. al., 2003).

#### Phytochemical screening: Chemical derivatization

The developed chromatographic plates were derivatize with  $\text{FeCl}_3$  to assess polyphenolic compound with p-anisaldehyde/sulphuric acid to visualize sterols and tri-terpenoids and with DPPH to detect the antioxidant activity by visualizing the colour change. P-anisaldehyde/ sulphuric acid is a universal reagent for derivatization of natural product to detect wide range of phytochemical compound. After derivatization with p-anisaldehyde reagent HPTLC chromatogram developed different colouring spot of light grey, purple-violet and reddish brown, which confirmed the

presence of different polyphenolic group and was best observed under 366nm and 416nm compare to 254nm. The colour of the carbohydrate band is sometimes similar to the monoterpenes band but after derivatization with sulphuric acid those band can be distinguished from each other as the carbohydrate band appeared as a pink colour (Ashraf et. al., 2021). The reddish brown and grey colour observed under 366nm (Figure 1A) which indicate the presence of triterpenes and steroid respectively. Greyish blue and purple colour was observed at 416nm (Figure 1A) which indicate the presence of monoterpenes. After derivatization, plates were heated for 10-15 min to increase the intensity of those spot. Neutralize ferric chloride solution for the detection of polyphenolic content of the plant *C. quadrangularis* was used as a derivatizing reagent, followed by scanning under 254nm, 366nm, 416nm. No such effective spot was visualized in HPTLC chromatogram after derivatization. Derivatization with DPPH showed multiple bands against purple background on the HPTLC plate at visible light 416 nm and 366nm (Figure 1B). Untreated DPPH, a free radical, shows purple colour band on the chromatographic plate. After reduction by the sample, it turned into non-radical form and visualized as yellow colour spot. Therefore, the free radical scavenging activity of the sample visualized as a yellow colour spot against a purple background and considered it as an antioxidant activity of the sample due to its reduction property. Our present study showed the presence of different polyphenolic group which has a major contribution on the antioxidant activity of the plant extract of *C. quadrangularis*.

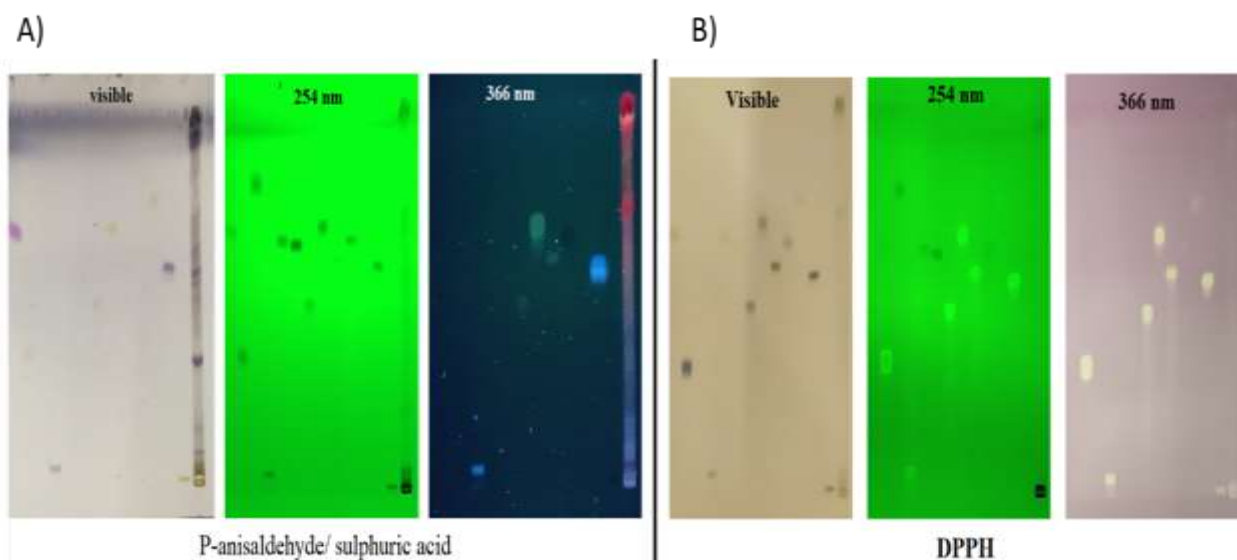


**Figure 1: HPTLC fingerprinting of extracts of *C. quadrangularis* with chemical derivatisation: A) white light after derivatization with p-anisaldehyde/sulfuric acid and B) white light after derivatization with DPPH•,**

#### Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of reference standard samples of different phenolic compound, some other phytochemicals and ethanolic extract are presented in Figure.2. The spectra of trans-cinnamic acid shows various characteristic peaks at 3066  $\text{cm}^{-1}$ , 3026  $\text{cm}^{-1}$ , 1681  $\text{cm}^{-1}$ , 1628  $\text{cm}^{-1}$ , 1420  $\text{cm}^{-1}$ , 1372  $\text{cm}^{-1}$ , and 1284  $\text{cm}^{-1}$  for stretching vibration of -OH group, -C-H alkene bond, -C=O, -C=C-, deformation of -O-H bond, phenolic -OH, and -C-O bond, respectively. The peaks at 768  $\text{cm}^{-1}$ , 682  $\text{cm}^{-1}$  and 589  $\text{cm}^{-1}$  correspond to the out-of-plane bending of -C-H bond of benzene-ring (Kustrina et. al., 2020). The spectrum of vanillic acid displays different peaks at 3484  $\text{cm}^{-1}$ , 1597  $\text{cm}^{-1}$ , 1522  $\text{cm}^{-1}$ , 1378  $\text{cm}^{-1}$ , 1111  $\text{cm}^{-1}$ , and 1028  $\text{cm}^{-1}$  for stretching vibration of -OH group, -C=O, aromatic ring, phenolic -OH, -C-O-C- and -C-C- and -C-H of benzene ring (Carvalho et. al., 2021). The spectrum of chlorogenic acid depicts different peaks at 3385  $\text{cm}^{-1}$ , 1686  $\text{cm}^{-1}$ , 1639  $\text{cm}^{-1}$ , 1442  $\text{cm}^{-1}$ , 1370  $\text{cm}^{-1}$  and 1289  $\text{cm}^{-1}$ , which correspond to the stretching vibration of -OH, -C=O, vinyl group, -C=C-, phenolic -OH and -C-O/-C-C- group (Hong et. al., 2008). Gallic acid displays the peaks at 3364  $\text{cm}^{-1}$ , 1618  $\text{cm}^{-1}$ , 1449  $\text{cm}^{-1}$ , 1246  $\text{cm}^{-1}$  and 1340  $\text{cm}^{-1}$ , which may be assigned to the stretching vibration of -OH, phenolic -OH, -C=O at aromatic ring, bending vibration of C-H of the benzene ring and phenolic -OH (Rui et. al., 2017). The spectrum of caffeic acid displays different peaks corresponding to stretching vibration

of -OH groups at 3399  $\text{cm}^{-1}$ , -C=O at 1642  $\text{cm}^{-1}$ , -C=O at 1597  $\text{cm}^{-1}$ , aromatic ring at 1446  $\text{cm}^{-1}$ , -C=C- of aromatic ring at 1351  $\text{cm}^{-1}$ , bending of phenolic ring at 1273  $\text{cm}^{-1}$ , phenolic -OH at 1212  $\text{cm}^{-1}$ , -C-C- at 892  $\text{cm}^{-1}$  and -C-H bending at 848  $\text{cm}^{-1}$  (Aydogdu et. al., 2019). The spectrum of p-coumaric acid shows characteristic peaks for stretching vibration of -OH groups, -C=O, -C=C-, aromatic -C-C-, phenolic -OH, -C-H, -C-OH, -C-C-O, and -C=O at 3341  $\text{cm}^{-1}$ , 1667  $\text{cm}^{-1}$ , 1625  $\text{cm}^{-1}$ , 1588  $\text{cm}^{-1}$ , 1372  $\text{cm}^{-1}$ , 1309  $\text{cm}^{-1}$ , 1282  $\text{cm}^{-1}$ , 976  $\text{cm}^{-1}$  and 688  $\text{cm}^{-1}$ , respectively (Nunes et. al., 2013).

The stretching vibration of -OH groups at 3286  $\text{cm}^{-1}$ , -C=O at 1669  $\text{cm}^{-1}$ , -C=C- at 1513  $\text{cm}^{-1}$ , phenolic-OH at 1373  $\text{cm}^{-1}$ , aromatic -C=O at 1313  $\text{cm}^{-1}$ , -C-O-C- at 1243  $\text{cm}^{-1}$  and aromatic ring at 931  $\text{cm}^{-1}$  were found in the spectrum of quercetin (Świsłocka et. al., 2012). The spectrum of apigenin displays different peaks at 3326  $\text{cm}^{-1}$ , 1652  $\text{cm}^{-1}$ , 1608  $\text{cm}^{-1}$ , 1373  $\text{cm}^{-1}$ , 1355  $\text{cm}^{-1}$ , 1245  $\text{cm}^{-1}$ , 1181  $\text{cm}^{-1}$  and 1032  $\text{cm}^{-1}$ , which may be assigned to stretching vibration of -OH group, -C=O group, -C=C- of aromatic ring, phenolic -OH group, benzene ring, Aryl-O, -C-O-, and -C-H of aromatic ring, respectively (Liu et. al., 2020). The spectrum of pure naringenin shows characteristic peaks at 3285  $\text{cm}^{-1}$ , 1601  $\text{cm}^{-1}$ , 1519  $\text{cm}^{-1}$ , 1375  $\text{cm}^{-1}$  and 1249  $\text{cm}^{-1}$ , which can be attributed to the stretching vibration of -OH groups, -C=O group, -C=C-, phenolic -OH group and -C-O-C-, respectively (Zhao et. al., 2017 & Nagula et. al. 2020).

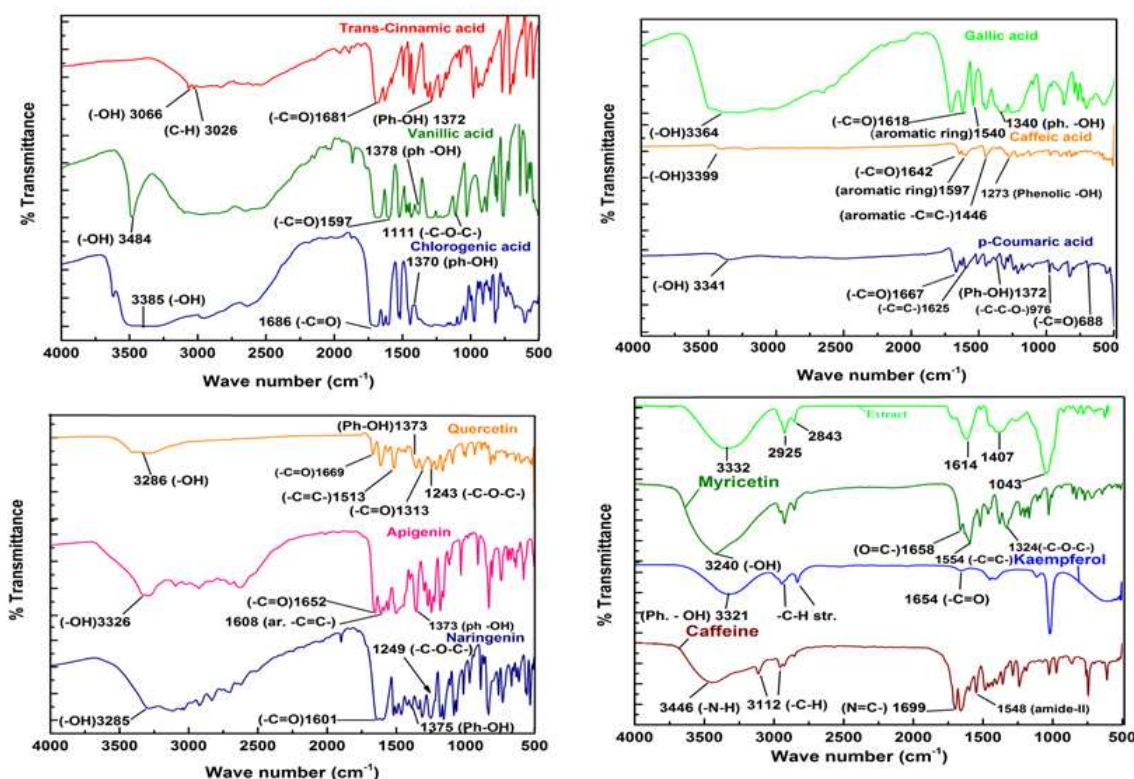


**Figure 2:** FTIR spectra of standard pristine sample of different phenolic acids along with the ethanolic and dichloromethane extracts obtain from *C. quadrangularis*

The spectra obtained from myricetin exhibits the peaks at  $3240\text{ cm}^{-1}$ ,  $1658\text{ cm}^{-1}$ ,  $1554\text{ cm}^{-1}$  and  $1324\text{ cm}^{-1}$  which may be assigned to stretching vibrations of -OH groups, -C=O, -C=C- and -C-O-C-, respectively (Gratieri et. al., 2020). The peaks at  $3321\text{ cm}^{-1}$ ,  $2944\text{ cm}^{-1}$ ,  $2832\text{ cm}^{-1}$ , and  $1654\text{ cm}^{-1}$  found in the spectra of kaempferol correspond to the stretching vibrations of phenolic -OH, -C-H, and -C=O, respectively (Han et. al., 2020 & Sharma, Sarin, 2012). The spectra obtained from pristine caffeine shows characteristic peaks at  $3446\text{ cm}^{-1}$ ,  $3112\text{ cm}^{-1}$ ,  $1699\text{ cm}^{-1}$ , and  $1548\text{ cm}^{-1}$  for stretching vibration of -N-H, -C-H, -C=N and amide-II (Nikoo et. al., 2018 & Nicoletti et. al., 2012).

#### Phytochemical screening: HPTLC fingerprinting

In the HPTLC chromatograms the position of the maximum peak denotes, the retention of the compound when compared with its reference substance. The sample can be visualized for identification in qualitative analysis and the intensity of the peak helps in quantitative analysis (Nile & Park, 2015). The use of this solvent system (Chloroform: Ethyl acetoacetate:

Formic Acid 5:4:1v/v) provides a good separation for the observed flavonoids and phenolics groups (Figure 1). Flavonoids like rutin, myricetin, quercetin, naringenin, apigenin, kaempferol and the phenolic acid like chlorogenic acid, gallic acid, P-coumaric acid, 4-hydroxybenzoic acid, trans-cinnamic acid, vanillic acid were used as a standard to identify and quantify of marker compound in the plant extract by comparing with the  $R_f$  value and by the area under the curve (AUC) of standard curve respectively. The HPTLC chromatogram exposed the presence of different spots. Among them were identified which implicate the existence of quercetin ( $R_f$ -0.6), myricetin ( $R_f$ -0.49), apigenin ( $R_f$ -0.68), gallic acid ( $R_f$ -0.35). The chromatogram of *C. quadrangularis* showed the bands prominently under 366nm, 254nm and visible light separated out before derivatization (Figure: 3A). The concentration of identified compounds were calculated from the regression equation which is derived from area under the curve of the plant extract and calculated value were in the figure 3C & 3B respectively (Johansen et. al., 2005).

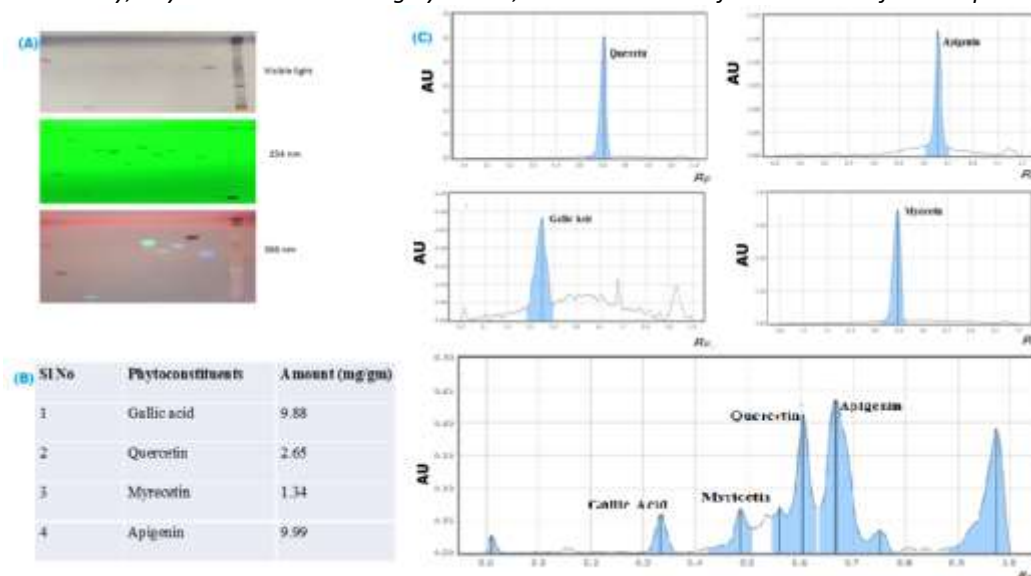


Figure 3A: HPTLC fingerprinting for extract *C. quadrangularis* under visible light, UV 254nm and UV 366nm. Mobile phase Chloroform: Ethyl

**acetoacetate:** Formic Acid at a ratio 5:4:1v/v. Track 1 to 13 with standard compounds are p- coumaric acid ; gallic acid ; trans cinnamic acid ; chlorogenic acid; vanillic acid ; 4-hydroxy benzoic acid ; myricetin ; quercetin; apigenin ; naringenin ; caffeic acid ; rutin and extract respectively. Figure 3B: Amount of identified compounds. Figure 3C: HPTLC Chromatogram of the standard flavonoids, phenolic compound and extract.

#### Phytochemical screening: GC-MS Analysis

GC-MS study was undertaken to find out the bioactive compounds present in the ethanolic extract of *C. quadrangularis* by using Gas chromatography and Mass spectroscopy. The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (peak area %) are presented in Table 3 which

shows the presence of forty-seven bioactive phytochemical compounds in the ethanolic extract of *C. quadrangularis*. The mass spectra of identified compounds from *C. quadrangularis* were presented Figure no. 4. The GC-MS profiling shows the presence of total 47 compounds among which eight compounds which have higher % area in the chromatogram like n-Hexadecanoic acid, Phytol, Z-7-Tetradecenal, Tetracosanoic acid, methyl ester, Squalene, gamma-Tocopherol, Vitamin E signifies the major portion in the extract. On the other hand, various compounds like gamma sitosterol, campesterol, pentadecanoic acid, methyl ester, Neophytadiene, 6,10,14-Trimethyl-2-pentadecanone, Hexadecanoic acid, 9-Hexadecen-1-ol, n-Hexadecanoic acid, Phytol, n-Tetracosanol-1, Tetracosanoic acid, Squalene and Stigmast-4-en-3-one was also identified.

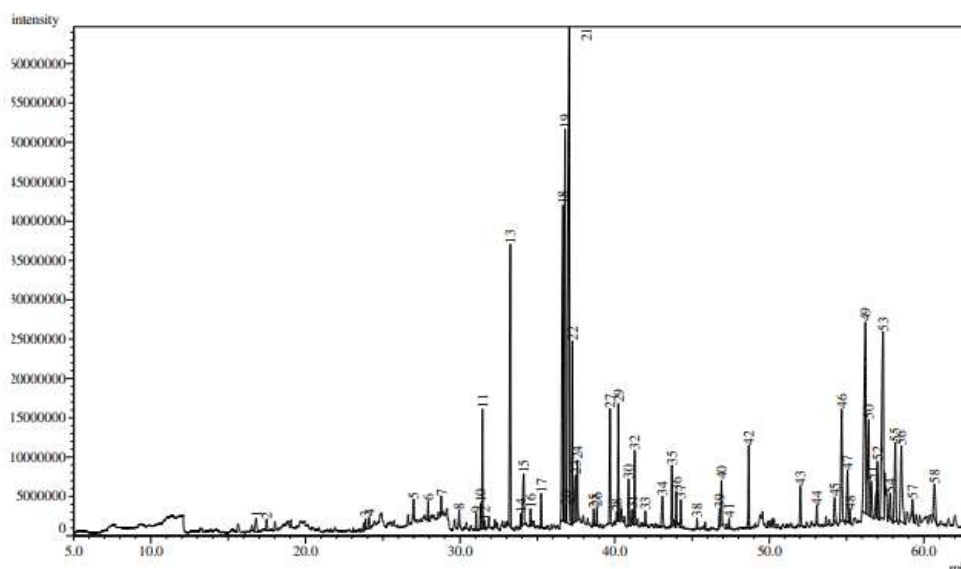


Figure 4: GC-MS chromatogram of ethanolic extract of *C. quadrangularis*

Table 3: Phytochemical constituents present in the ethanolic extract of *C. quadrangularis*

| Sl no | RT (min) | Name of compound                                                   | Molecular formula | Molecular weight | Peak area % | Reported/ Not reported |
|-------|----------|--------------------------------------------------------------------|-------------------|------------------|-------------|------------------------|
| 1     | 16.802   | 2,5-Monomethylene-l-rhamnitol                                      | C7H14O5           | 178              | 0.50        | Not reported           |
| 2     | 17.479   | Indole                                                             | C8H7N             | 117              | 0.28        | Reported               |
| 3     | 23.885   | Dodecanoic acid, methyl ester                                      | C13H26O2          | 214              | 0.14        | Reported               |
| 4     | 26.988   | 7-(1-Hydroxy-cyclohex-2-enyl)-2,2-dimethyl-hept-5-en-3-one         | C15H24O2          | 236              | 0.33        | Not reported           |
| 5     | 28.779   | Methyl tetradecanoate                                              | C15H30O2          | 242              | 0.23        | Reported               |
| 6     | 29.937   | 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one | C11H16O3          | 196              | 0.23        | Not reported           |
| 7     | 31.038   | Pentadecanoic acid, methyl ester                                   | C16H32O2          | 256              | 0.23        | Reported               |
| 8     | 31.323   | Neophytadiene                                                      | C20H38            | 278              | 0.37        | Reported               |
| 9     | 31.441   | 6,10,14-Trimethyl-2-pentadecanone                                  | C18H36O           | 268              | 1.83        | Reported               |
| 10    | 31.583   | 3,7,11,15-Tetramethylhexadecanol                                   | C20H42O           | 298              | 0.15        | Reported               |
| 11    | 33.246   | Hexadecanoic acid, methyl ester                                    | C17H34O2          | 270              | 5.39        | Reported               |
| 12    | 33.922   | 9-Hexadecen-1-ol, (Z)                                              | C16H32O           | 240              | 0.20        | Not reported           |
| 13    | 34.113   | n-Hexadecanoic acid                                                | C16H32O2          | 256              | 1.49        | Reported               |
| 14    | 35.247   | Hexadecanoic acid, 15-methyl-, methyl ester                        | C18H36O2          | 284              | 0.45        | Reported               |
| 15    | 36.650   | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester                     | C19H34O2          | 294              | 8.42        | Reported               |
| 16    | 36.792   | 6-Octadecenoic acid, methyl ester                                  | C19H36O2          | 296              | 9.08        | Reported               |
| 17    | 36.858   | 11-Octadecenoic acid, methyl ester                                 | C19H36O2          | 296              | 0.15        | Not reported           |
| 18    | 37.064   | Phytol                                                             | C20H40O           | 296              | 17.36       | Reported               |
| 19    | 37.264   | Methyl stearate                                                    | C19H38O2          | 298              | 2.82        | Reported               |
| 20    | 37.468   | 10E,12Z-Octadecadienoic acid                                       | C18H32O2          | 280              | 1.87        | Reported               |
| 21    | 37.583   | Z-7-Tetradecenal                                                   | C14H26O           | 210              | 1.39        | Reported               |
| 22    | 38.610   | Oxirane, tetradecyl                                                | C16H32O           | 240              | 0.22        | Not reported           |
| 23    | 39.695   | Undec-10-ynoic acid, tetradecyl ester                              | C25H46O2          | 378              | 1.91        | Not reported           |
| 24    | 40.125   | 2-(Dimethylamino)ethyl vaccenoate                                  | C22H43NO2         | 353              | 0.16        | Not reported           |
| 25    | 40.893   | Methyl 18-methylnonadecanoate                                      | C21H42O2          | 326              | 0.87        | Not reported           |
| 26    | 41.291   | 4,8,12,16-Tetramethylheptadecan-4-olide                            | C21H40O2          | 324              | 1.21        | Reported               |
| 27    | 43.087   | 2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13,14-octahydro-6-     | C17H24O4          | 292              | 0.78        | Not reported           |

|    |        |                                                                                                             |          |     |      |              |
|----|--------|-------------------------------------------------------------------------------------------------------------|----------|-----|------|--------------|
|    |        | oxabenzocyclododecen-5-one                                                                                  |          |     |      |              |
| 28 | 43.699 | Behenic alcohol                                                                                             | C22H46O  | 326 | 0.19 | Not reported |
| 29 | 43.982 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester                                                   | C19H38O4 | 330 | 0.50 | Reported     |
| 30 | 44.274 | Docosanoic acid, methyl ester                                                                               | C23H46O2 | 354 | 0.49 | Reported     |
| 31 | 45.326 | n-Tetracosanol-1                                                                                            | C24H50O  | 354 | 0.14 | Reported     |
| 32 | 46.788 | 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester                                                 | C21H38O4 | 354 | 0.31 | Reported     |
| 33 | 46.909 | 9-Hexacosene                                                                                                | C26H52   | 364 | 0.96 | Not reported |
| 34 | 47.402 | Tetracosanoic acid, methyl ester                                                                            | C25H50O2 | 382 | 0.11 | Not reported |
| 35 | 48.669 | Squalene                                                                                                    | C30H50   | 410 | 1.17 | Reported     |
| 36 | 52.008 | gamma.-Tocopherol                                                                                           | C28H48O2 | 416 | 0.78 | Reported     |
| 37 | 53.072 | Vitamin E                                                                                                   | C29H50O2 | 430 | 0.31 | Reported     |
| 38 | 54.684 | Campesterol                                                                                                 | C28H48O  | 400 | 2.90 | Reported     |
| 39 | 55.057 | Stigmasterol                                                                                                | C29H48O  | 412 | 1.13 | Reported     |
| 40 | 56.133 | 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane | C33H56   | 452 | 0.33 | Not reported |
| 41 | 56.220 | gamma.-Sitosterol                                                                                           | C29H50O  | 414 | 7.39 | Reported     |
| 42 | 56.594 | beta.-Amyrin                                                                                                |          |     | 1.12 | Reported     |
| 43 | 57.357 | Lup-20(29)-en-3-one                                                                                         | C30H48O  | 424 | 7.44 | Reported     |
| 44 | 57.816 | Lupeol                                                                                                      | C30H50O  | 426 | 0.68 | Reported     |
| 45 | 58.543 | Stigmast-4-en-3-one                                                                                         | C29H48O  | 412 | 2.84 | Reported     |
| 46 | 60.683 | Friedelan-3-one                                                                                             | C30H50O  | 426 | 1.25 | Reported     |

**In Vitro antioxidant study****Total Phenolic and flavonoid content**

Total phenolic content and total flavonoid content of the plant can be correlated with the standard compounds like gallic acid and quercetin respectively.

Here total phenolic content and total flavonoids content were estimated 20.46±0.01 mg/g equivalent to Gallic acid and 34.8±0.014 mg/g equivalent to quercetin respectively in Table 4.

**Table 4. Total Phenolic and flavonoid content of the extract of the plant *C. quadrangularis***

| Sl No | Experiments             | Result (mg/g) |
|-------|-------------------------|---------------|
| 1     | Total Phenolic content  | 20.46±0.01    |
| 2     | Total flavonoid content | 34.8±0.014    |

**DPPH radical scavenging activity**

The DPPH scavenging activity of the plant extract as well as ascorbic acid was shown in the Figure 5. IC<sub>50</sub> value was measured by comparing the absorbance of different concentration of test sample with the standard curve of Ascorbic acid. The IC<sub>50</sub> value of ethanolic extract of *C. quadrangularis* is 49.0±0.12 mcg/ml compared to IC<sub>50</sub> value of ascorbic acid 38.5±0.25 mcg/ml. The concentration with less value of IC<sub>50</sub> is capable of showing good radical scavenging activity.

**Determination of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) radical scavenging activity**

The ability of the *C. quadrangularis* extract to scavenge hydrogen peroxide was determined according to the method of Ruch et al. The scavenging ability of *C. quadrangularis* extract on hydrogen peroxide is shown in Figure. 5B and compared with ascorbic acid as standards. The *C. quadrangularis* extract was capable of scavenging hydrogen peroxide in an amount dependent manner. Figure. 5B shows that the *C. quadrangularis* extract had strong hydrogen peroxide scavenging activity with IC<sub>50</sub> 64.5±0.25 mcg/ml (Table: 5B).

**Superoxide anion scavenging activity**

In the NADH-NBT system, NBT is reduced by the superoxide anion that is produced from dissolved oxygen through the NADH coupling mechanism. Thus, the consumption of superoxide anion in the reaction mixture is indicated by the decrease in absorbance at 560 nm with antioxidants. The % suppression of superoxide radical formation of 50–500 mg of *C. quadrangularis* ( $IC_{50} 122.1 \pm 0.31$  mcg/ml) extract is shown in Figure 5C, along with a comparison with the same doses of Ascorbic Acid ( $IC_{50} 90.5 \pm 0.51$  mcg/ml) (Table 4).

**ABTS scavenging activity**

Figure 5D illustrates how *C. quadrangularis* extract scavenges ABTS radicals in a concentration-dependent manner (25–250 µg/ml) in comparison to Ascorbic Acid. The outcomes demonstrated that the *C. quadrangularis* extract outperformed Ascorbic Acid in terms of ABTS radical scavenging activity and possessed superior ABTS radical scavenging ability. Figure 5D shown  $IC_{50}$  value of *C. quadrangularis* extract and Ascorbic Acid  $62.2 \pm 0.32$  mcg/ml and  $89.1 \pm 0.32$  mcg/ml respectively.

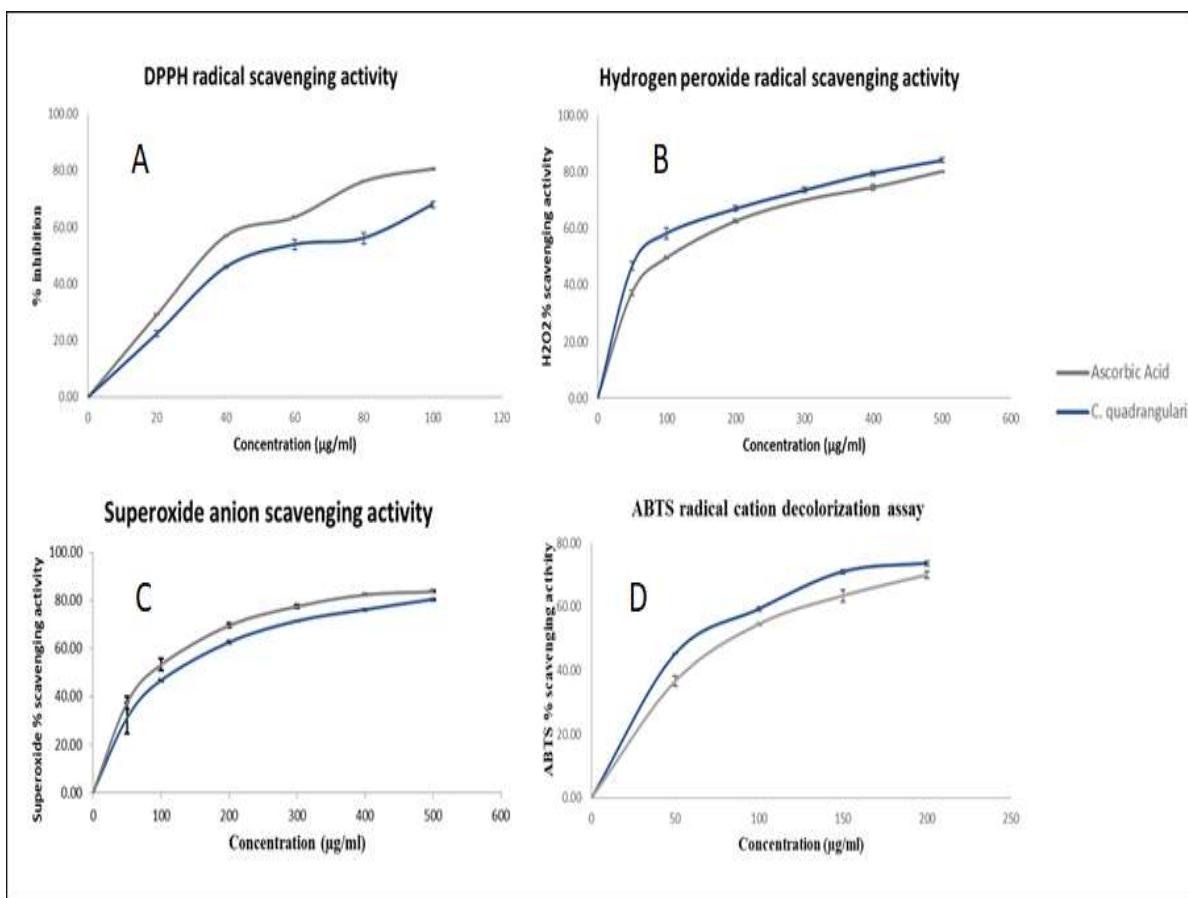


**Figure 5: Antioxidant activity of the *C. quadrangularis* extract at various concentrations. (A) DPPH radical scavenging activity, (B)  $H_2O_2$  radical scavenging activity, (C) Superoxide radical scavenging activity, (D) 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity. The data were expressed as mean  $\pm$  SD,  $n=3$ . The significance was considered when  $p < 0.05$**

**Table 5: List of  $IC_{50}$  Value of *C. quadrangularis* extract**

| Plant Standard                   | Extract/ | $IC_{50}$ Value (mcg/ml) of different oxidant radicals |                  |                    |                 |
|----------------------------------|----------|--------------------------------------------------------|------------------|--------------------|-----------------|
|                                  |          | DPPH radical                                           | $H_2O_2$ radical | Superoxide radical | ABTS radical    |
| Ascorbic acid                    |          | $38.5 \pm 0.25$                                        | $100.0 \pm 0.31$ | $90.5 \pm 0.51$    | $89.1 \pm 0.32$ |
| <i>C. quadrangularis</i> extract |          | $49.0 \pm 0.12$                                        | $64.5 \pm 0.25$  | $122.1 \pm 0.31$   | $62.2 \pm 0.34$ |

**Determinations of antibacterial activity of crude extract *C. quadrangularis*.**

The extracts of the studied plants exhibited varying degrees of inhibition activity against two gram negative and two-gram positive tested bacteria and the results were expressed in terms

of the diameter of the growth-inhibition zone (clear zones). The results clearly showed that tested bacteria were susceptible to extracts. Ethanolic crude extract showed high activity

*Antimicrobial activity, Phytochemical screening by HPTLC, FTIR and GC-MS of the extracts of Cissus quadrangularis (L.)*

against *E. coli* (ATCC 10231) and *S typhi* (NCTC-786) and moderate activity against *S. aureus*-ATCC-9144 (ATCC-9144) and *B. subtilis* (ATCC 9372) which is also similar in activity to Ampicillin Figure 6.

The extract *C. quadrangularis* was tested against gram-negative bacteria, *Salmonella typhi* (NCTC-786) and *E. coli* (ATCC 10231) and gram-positive bacteria, *Bacillus subtilis* (ATCC 9372) and *Staphylococcus aureus* (ATC C-9144).

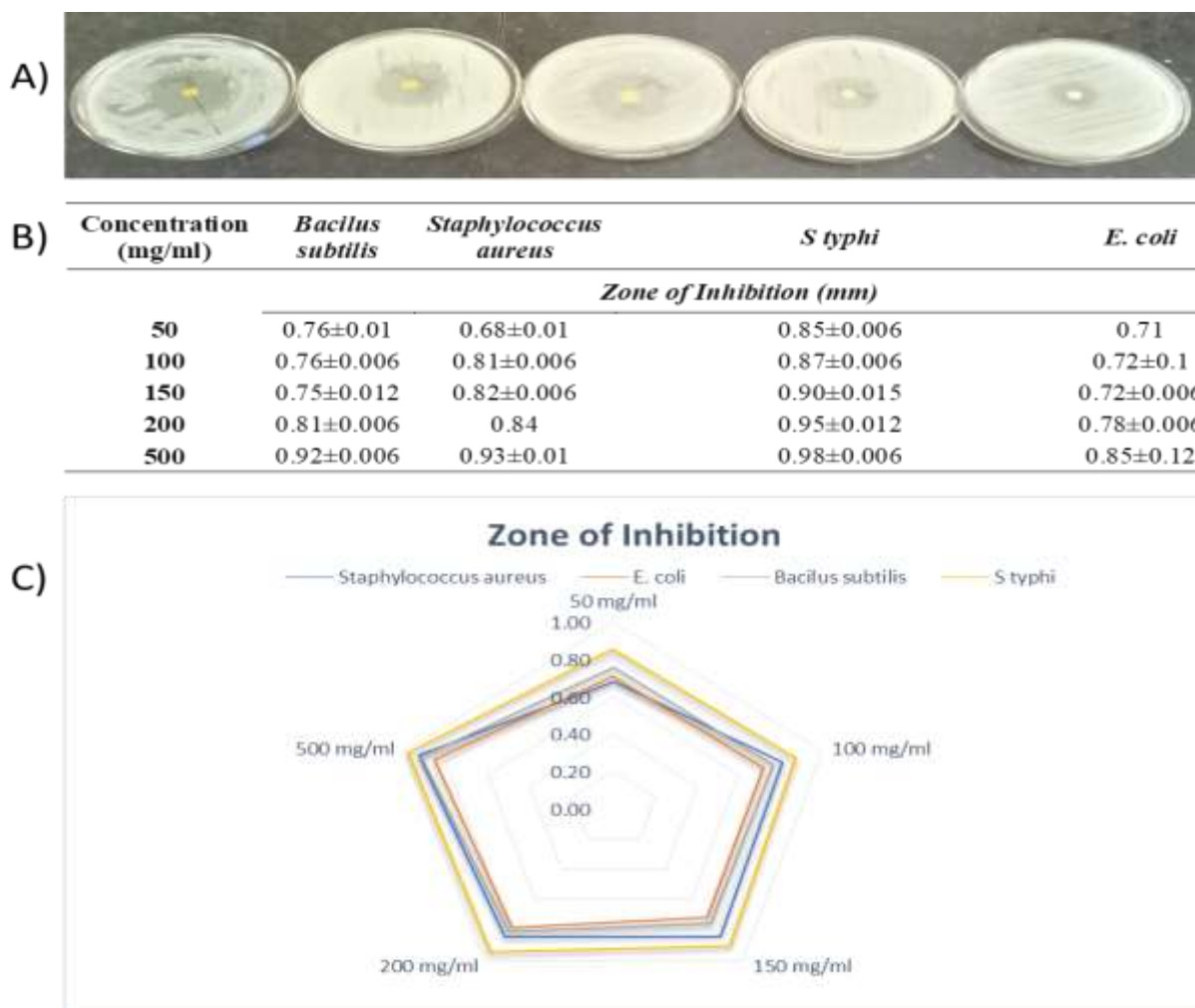


Figure 6A: Picture of zone of inhibition of Gram-negative bacteria (*E. coli*-ATCC 10231) agents at various different concentrations of extracts of *C. quadrangularis* and Ampicillin (std). Figure 6B:

**Data of Zone of Inhibition of Gram-negative bacteria (*E. coli*-ATCC 10231 and *S typhi* NCTC-786) and Gram-positive bacteria (*S. aureus*-ATCC-9144 and *B. subtilis*-ATCC 9372) agents at various different concentrations of extracts of *C. quadrangularis*. Figure 6C: Radar charts of results analysis of zone of inhibition.**

#### Determination of docking scores and MM-GBSA energy

The molecular grids for 1AJ6 and 7D6G were validated by re-docking the bound inhibitors to the binding site and determining the RMSD values between the crystal pose and docked pose generated by Glide XP. Generally, RMSD values  $\leq 2.0$  Å indicate a good correlation between the native binding pose and the pose generated by the docking algorithm. The RMSD values between the native and docked poses of novobiocin and folate were 0.463 Å and 1.570 Å, respectively,

indicating a good correlation between the native and docked poses.

The results of molecular docking revealed that the docking scores and MM-GBSA energies of all the three compounds to 1AJ6 were inferior to those of the bound inhibitor, novobiocin (Table 1). However, the ligand efficiency of ampicillin was superior to that novobiocin. The results of MM-GBSA analysis revealed that the binding affinities of the three compounds to 1AJ6 were comparable, but lower than that of novobiocin. The docking score of the bound ligand (folate) to 7D6G was superior to those of the three compounds. The binding affinity of gamma sitosterol to 7D6G was highest among the three compounds and also higher than that of folate. The results further revealed that the docking scores of ampicillin were superior to those of the other compounds for all the targets.

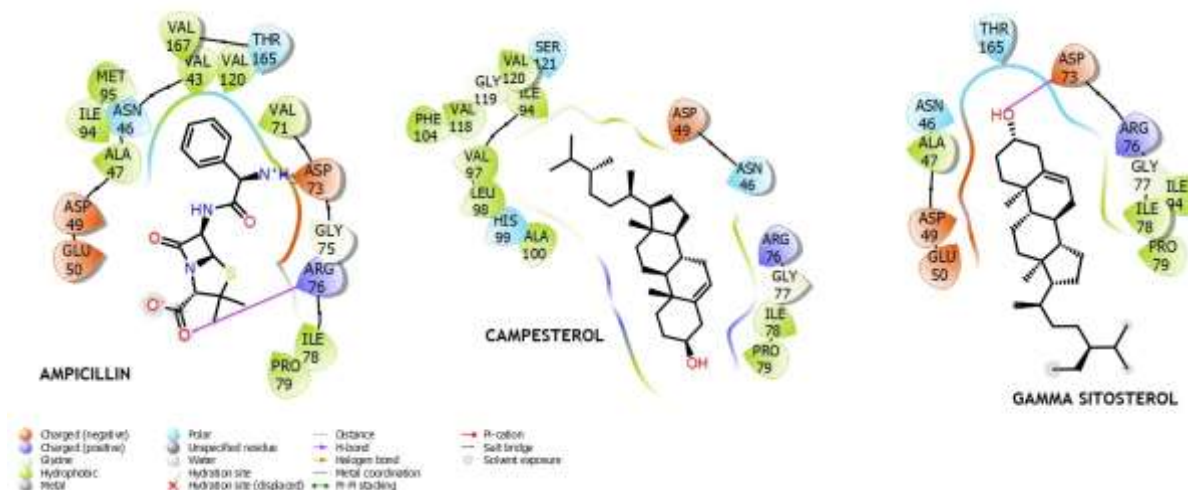
**Table 6: Docking scores and MMGBSA energy of the two compounds against the 3 targets**

| Target (PDB ID) | Compounds                    | Glide XP docking score | Ligand efficiency | MM-GBSA energy (kCal/mol) |
|-----------------|------------------------------|------------------------|-------------------|---------------------------|
| 1AJ6            | Novobiocin (bound inhibitor) | -7.272                 | -0.165            | -60.30                    |
|                 | Ampicillin                   | -6.296                 | -0.262            | -26.99                    |
|                 | Campesterol                  | -3.768                 | -0.130            | -26.82                    |
|                 | Gamma sitosterol             | -2.851                 | -0.095            | -29.43                    |
| 7D6G            | Folate (bound ligand)        | -11.260                | -0.352            | -80.07                    |
|                 | Ampicillin                   | -6.524                 | -0.272            | -48.62                    |
|                 | Gamma sitosterol             | -5.546                 | -0.185            | -81.89                    |
|                 | Campesterol                  | -4.987                 | -0.172            | -67.98                    |
| 1GG4            | Ampicillin                   | -4.775                 | -0.199            | -34.40                    |
|                 | Campesterol                  | -3.724                 | -0.128            | -22.67                    |
|                 | Gamma sitosterol             | -3.511                 | -0.117            | -32.97                    |

**Interaction analysis**

Analysis of the protein-ligand interactions revealed that ampicillin and gamma sitosterol formed one hydrogen bond with 1AJ6, and the campesterol-1AJ6 complex was primarily stabilised by hydrophobic interactions. The protein-ligand hydrogen bonds were mediated via Glu 50, Asp 73, and Arg 76 (Figure 7). Ampicillin formed six hydrogen bonds with residues Thr 112, Ser 113, His 281, Asn 282, Arg 316, and Leu 317 in the binding site of 1GG4 (Figure 8). Campesterol formed one hydrogen bond with Lys 311, while gamma sitosterol formed one hydrogen bond each with Glu 116.

Analysis of the protein-ligand interactions of 7D6G revealed that ampicillin formed only one hydrogen bond with Arg 52 in the binding site, while the other two compounds did not form any hydrogen bonds with the binding site of 7D6G. The ligand-protein complexes were also stabilised by hydrophobic interactions with the binding site residues (Figure 9). Altogether, the results of protein-ligand interaction analysis revealed that of the three compounds, ampicillin formed the highest number of hydrogen bonds with the binding sites of the three targets.

**Figure 7: Interactions of ampicillin, campesterol, and gamma sitosterol with the PDB structure 1AJ6, as determined by molecular docking.**

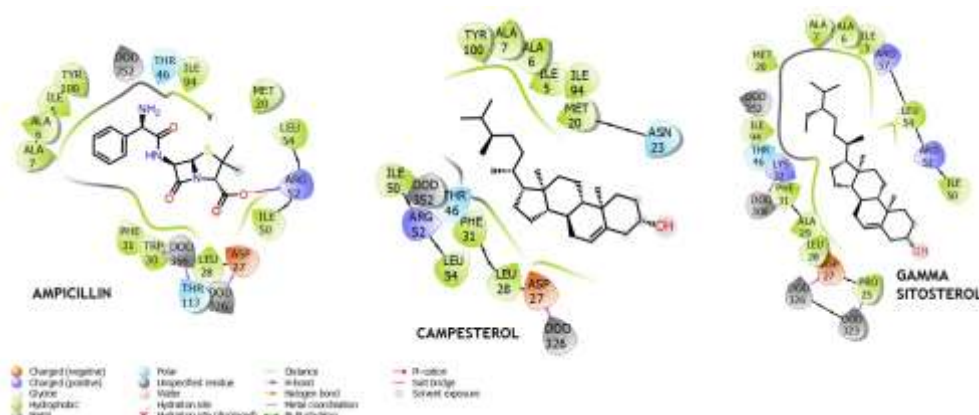


**Figure 9: Interactions of ampicillin, campesterol and gamma sitosterol with the PDB structure 7DG6, as determined by molecular docking.**

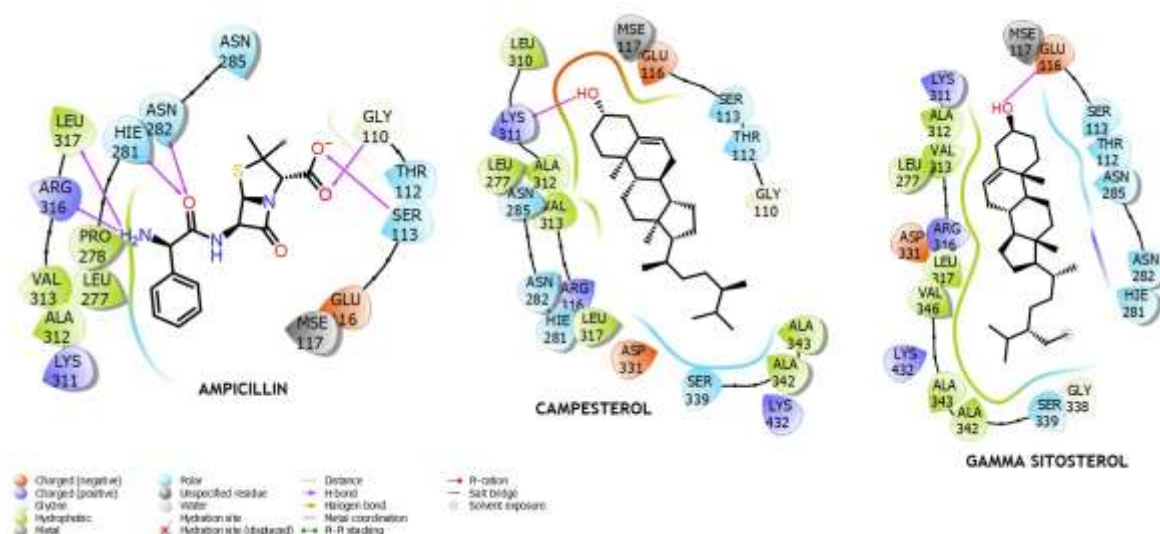


**Figure 8: Interactions of ampicillin, campesterol, and gamma-sitosterol with the PDB structure 1GG4, as determined by molecular docking.**

## Discussion

To address the critical issue of growing bacteria resistance against conventional antibiotics, it is vital to find novel antimicrobial chemicals or extracts. Biodiversity in plant chemicals is a rich potential resource. Finding the active components of *C. quadrangularis* that exhibit antibacterial action is the aim of this investigation. The preliminary phytochemical screening revealed some significant active compounds such as reducing sugar, glycoside, flavonoids, and alkaloids (Table 2). The IR spectra obtained from the ethanolic extract shows almost all the characteristic peaks of gallic acids, quercetin, myricetin and apigenin, which indicates their presence in the extract. The results of FTIR study were also substantiated by the HPTLC analysis. By the HPTLC study, quercetin, myricetin, apigenin and gallic acid compounds are identified and quantified (Figure 3). Total phenolic content and total flavonoid content of the plant extract  $20.46 \pm 0.01$  mg/g equivalent to Gallic acid and  $34.8 \pm 0.014$  mg/g equivalent to quercetin is shown respectively in Table 4. The plant extract has a total phenolic content of  $20.46 \pm 0.01$  mg/g, which is equivalent to gallic acid, and a total flavonoid content of  $34.8 \pm 0.014$  mg/g, which is

equivalent to quercetin. The correlation of the results between the antioxidant activity and contents of bioactive phytochemicals in extract may have synergistic effect. Following an investigation, it was discovered that the extract exhibits antibacterial effects against *E. coli* ATCC 10231, *S. aureus*-ATCC-9144, *Salmonella typhi* - NCTC-786, and *S. aureus*-ATCC-9144. By using GC-MS analysis, numerous compounds were found. The identification of various substances with strong antioxidant capabilities, such as n-Hexadecanoic acid, Phytol, Z-7-Tetradecenal, Tetracosanoic acid, methyl ester, Squalene, Tocopherol, and Vitamin E, was made. On the other hand, several substances that have antimicrobial activity have been discovered, including pentadecanoic acid, methyl ester, n-tetracosanol-1, tetracosanoic acid, squalene, and stigmast-4-en-3-one, as well as hexadecanoic acid, 9-hexadecen-1-ol, and n-hexadecanoic acid. Numerous inflammatory processes associated with chronic inflammatory disorders, such as heart dysfunction, neurological diseases or diabetes, include oxidative stress (Johansen et. al., 2005). *Cissus quadrangularis*, a traditional medicinal plant, shows promise in treating chronic inflammatory disorders due to its potent anti-inflammatory,

antioxidant, and analgesic properties. Key applications include managing conditions like arthritis, inflammatory bowel disease (IBD), and asthma. Its bioactive compounds, such as flavonoids, steroids, and triterpenoids, help reduce inflammation by inhibiting key inflammatory mediators like TNF- $\alpha$  and IL-6. Additionally, it can aid in bone health and repair, crucial for conditions like rheumatoid arthritis. Its antioxidant activity mitigates oxidative stress, a significant factor in chronic inflammation, thus potentially reducing disease progression and symptom severity. Preliminary studies and traditional usage suggest that *Cissus quadrangularis* may offer a natural, complementary approach to conventional anti-inflammatory therapies, though more extensive clinical research is needed to fully validate its efficacy and safety. Many phenolic substances, including flavonoids, isoflavones, and phenolic acids, have demonstrated antioxidant action. Number of studies have revealed the advantages of antioxidant phenolic compounds derived from natural sources in the prevention of chronic illnesses (Sies et. al., 2012 & Unnikrishnan et. al., 2014). Flavonoids, phenolic acid, and tannins are phenolic substances that significantly increase the antioxidant properties of medicinal plants. After going through the examinations for total phenolic, total flavonoids, and DPPH scavenging, it is observed that the plant extract possesses strong anti-oxidant property.

Here before analysis of antioxidant activity, phytochemical analysis was done by different chromatographic techniques. The GC-MS analysis revealed some phytochemicals like n-Hexadecanoic acid (Butt et. al., 2004 & Avoseh et. al., 2021), Phytol, Z-7-Tetradecenal (Wei et. al., 2015), Tetracosanoic acid methyl ester, gamma-Tocopherol, Vitamin E (Shaaban, Ghaly & Fahmi, 2021) which have established antioxidant, and Pentadecanoic acid, methyl ester, Neophytadiene, 6,10,14-Trimethyl-2-pentadecanone, Hexadecanoic acid (Steck et. al., 1982), 9-Hexadecen-1-ol, n-Hexadecanoic acid, Phytol, n-Tetracosanol-1, Tetracosanoic acid, Squalene and Stigmast-4-en-3-one which also have established antioxidant and antimicrobial activity respectively (Salem et. al., 2017; Xu et. al., 2016; Hilmarsson et. al., 2005). For instance, the anti-inflammatory (Hattori et. al., 1987), antioxidant, hypocholesterolemic (Ganesan et. al., 2022), antibacterial (Aparna et. al., 2012), activities reported for n-hexadecanoic acid (palmitic acid). Some other phyto-constituents also identified by the GC-MS analysis (Kumar et. al., 2010), those have anticancer, antidiabetic, anti-inflammatory, hepatoprotective activity, antinociceptive, gastro-protective etc (Rahuman et. al., 2000; Krishnamoorthy et. al., 2014). Out of forty-seven compounds that have been identified by GC-MS analysis, total fourteen compounds have not been reported yet in any literature. The non-reported compounds are 2,5-Monomethylene-l-rhamnitol, 7-(1-Hydroxy-cyclohex-2-enyl)-2,2-dimethyl-hept-5-en-3-one, 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one, 9-Hexadecen-1-ol, (Z), 11-Octadecenoic acid, methyl ester, Oxirane, tetradecyl, Undec-10-ynoic acid, tetradecyl ester, 2-(Dimethylamino)ethyl vaccenoate, Methyl 18-methylnonadecanoate, 2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13,14-octahydro-6-oxabenzocyclododecen-5-one, Behenic alcohol, 9-Hexacosene, Tetracosanoic acid, methyl ester, 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-

tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane.

Treatments with traditional antibiotics are becoming less effective as the prevalence of bacteria increases at an alarming rate. *Salmonella typhi*-NCTC-786, *S. aureus*-ATCC-9144, and *E. coli*-ATCC 10231, which cause infections of the urinary, respiratory, and reproductive tracts in animals, are examples of multi-resistant bacteria (Rajeswari et. al., 2013). In our experiment the bacterial strains of two gram positive and two-gram negative bacteria were tested by disc diffusion method. Among the bacterial strains, gram (-ve) bacteria gives more performed significant zone of inhibition activity than the gram (+ve) bacteria.

Analysis of the docking results revealed that the ampicillin, gamma sitosterol, and campesterol formed several hydrogen bonds with the three targets. The hydrogen bonds formed by the two identified compounds (gamma sitosterol and campesterol) and the standard antibiotic ampicillin were comparable to those formed by the standards, ligands like novobiocin and folate, with 1AJ6 and 7D6G, respectively. It is observed from the docking study that campesterol and gamma sitosterol have strong binding affinity towards 7D6G (Folate reductase) which is essential for folic acid synthesis and folic acid is one of the important factors for bacterial cell wall. On the other hand, effect of these two compounds on 1AJ6 (Topoisomerase) enzyme is required for prevention of unwinding of the DNA supercoils which results in inhibiting the further DNA replication. Now, when these two compounds will act together on these enzymes, it will show the synergistic or additive effect to prevent bacterial growth further. Additionally, the results of MM-GBSA energy analysis revealed that the binding affinity of gamma sitosterol to the active site of 7D6G was much higher than that of folate. Altogether, these findings suggested these two phytochemicals may play a significant role in producing the antimicrobial activity. In future, the identified compounds can be isolated by utilizing column chromatography and those compounds can further be tested in in-vitro and in-vivo animal model to accurately confirm their claimed effects.

## Conclusion

The present study shows that the extract of *C. quadrangularis* contains various potent Phyto-constituents includes four phenolic compounds like quercetin, myricetin, apigenin and gallic acid which has been confirmed by FTIR study. The study has shown the presence of functional groups associated with these phenolic compounds. The GC-MS analysis has also identified forty-seven compounds among which few have been already found in *C. quadrangularis*. But few compounds like Undec-10-ynoic acid tetradecyl ester, 9-Hexacosene, Methyl-18-methylnonadecanoate have been identified from *C. quadrangularis* by this study. The extract of the stem of the plant exhibits various pharmacological activities like anti-oxidant, anti-inflammatory, anti-diabetic, gastro-protective, hepato-protective activities which can be confirmed by further in-vivo animal studies. Thus *C. quadrangularis* can be further studied and can be a promising natural source for various chronic disorders.

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Declaration of competing interest

The researchers claim no conflict of interests.

## Credit authorship contribution statement

Shubham Paul, Sakshar Saha, Ritu Khanra: designed the experiments, supervised and participated entire work. Atanu Chatterjee: contributed to the writing of this manuscript, Srijan Panigrahi, Dipanjan Sengupta: performed antimicrobial study. Ranabir Sahu: performed HPTLC study. Gouranga Nandi: IR data interpretation, Achintya Saha, Aditi Gangopadhyay: Molecular docking analysis and interpretation.

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