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# ANALYSIS OF POTENTIAL TOXICOLOGICAL, PHYTOCHEMICAL AND ANTICANCER PROPERTIES FROM *CISSUS QUANDRANGULARIS*

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**ABSTRACT:** Present study was aimed to evaluate the preliminary pharmacognosy properties such as antimicrobial, antioxidant and anti-cancer potential of ethanol extract of *Cissus quadrangularis*. Antimicrobial activity was tested against *Staphylococcus aureus, Bacillus subtilis, Vibrio cholera, Vibrio parahaemolyticus, Pseudomonas aeruginosa*, *Entero toxigenic Excherichia coli (ETEC) and Salmonella typhi* by disc diffusion assay. Antioxidant activity was determined by DPPH free radical scavenging assay and ABTS free radical scavenging assay. MCF-7 breast cancer cells were used to investigate the in vitro anti-cancer activity of ethanol extracts of cissus. The anticancer activity MTT assay carried out using MCF7 cell line. The results CQE extract showed more activity against *Vibrio parahaemolyticus, Vibrio cholera, ETEC* and *Salmonella typhi* at 5–25 mg/well. The extract also exhibited dose dependent scavenging of DPPH and ABTS radicals. DPPH radicals was more efficient than that of ABTS radicals as revealed by the low IC50 value. MCF-7 breast cancer cells were used to investigate the in vitro anti-cancer activity of ethanol extracts of Cissus. The MTT assay results showed considerable anti-cancer activity in MCF–7 cancer cells, with an IC50 value of 40 µg/ml. Cissus extract effectively inhibited proliferation of MCF-7 cells in a dose-dependent manner over 24h. Thus the compounds of *Cissus quadrangularis* displayed potential for antioxidant, antibacterial and anti-cancer agents.

**KEYWORDS:** Cissus Quadrangularis, Antibacterial, Anti-Oxidant, MCF–7.

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2018 May – June RJLBPCS 4(3) Page No.94

## 1.INTRODUCTION

Medicine in contemporary India is a fascinating blend of the traditional system with conventional one and often been used for various historical, cultural and ecological and socio-economical reasons [1,2]. In spite of enormous progress in the modern medical system, about 80% of the world population still depends on traditional systems of medicine for primary health care, which is true in Indian scenario also [3]. *Cissus quadrangularis* is a vining plant native to India and Africa that has been used medicinally for centuries [4-6]. The extensive list of applications of Cissus includes: bone fracture healing and tissue repair, prevention of osteoporosis, weight loss, blood sugar regulation, digestive disorders, menstrual irregularity, cholesterol and triglyceride lowering, antimicrobial, analgesic, antipyretic, anti-inflammatory, tissue protective, joint health, increased lean muscle mass and other folkloric applications. Traditionally, dried powders of the plant were used. However, alcoholic (ethanol or methanol) extracts are now widely used in the commercial market. All parts of the plant are claimed to be of therapeutic value. The use of this plant by the common folk for promoting fracture healing process is an old practice. In Ayurveda was the Indian traditional health care system [7]. Some of these historical uses of *C. quadrangularis L*., have been further investigated in recent years using scientific methods. In addition to its therapeutic uses, the young stem of the plant is used in making curries and chutneys for human consumption. The ash prepared from the plant is as a substitute for baking soda [8]. The USDA Agricultural Research Service has also listed uses of *C. quadrangularis L*. as ornamental, human food (vegetable) and medicinal (folklore). It has also been used as a tonic, an analgesic, and antihelmenthic medicine. The whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant are important in the administration of various ailments [910,11]. *C. quadrangularis L*., Phytochemical analyses showed high levels of carotene, anabolic teroidal substances, calcium and ascorbic acid. The stem of this plant has been reported to contain two asymmetric tetracyclic triterpenoids. The presence of b-sitosterol, d-amyrin, d-amyrone, and flavanoids (quercetin) has also been reported [12,13]. The unique chemical constituents of *C. quadrangularis* such as flavonoids and indanes, as well as phytosterols and keto-steroids, have shown promise as powerful and efficient antioxidants [14, 15]. The phytochemical analysis of the plant showed the presence of vitamin C, ß-carotene, two symmetric tetracycline triterpenoids, ß-sitosterol, α-amyrone. In addition to vitamin C, it also contains a high amount of carotene A, anabolic steroidal substance, and calcium [16].The methanolic extract of *Cissus quadrangularis* has been shown to inhibit iNOS activity in damaged gastric mucosa induced by aspirin [17]. Moreover, *Cissus quadrangularis* extract suppressed ear and paw edema in rats induced by ethyl phenylpropiolate and by both carrageenin as well as arachidonic acid, respectively [18]. The molecular mechanism and anti-inflammatory activity the molecular mechanism of the ethyl acetate extract of *Cissus quadrangularis* stem (CQE) in LPS-

stimulated RAW 264.7 macrophage cells. These findings provide the scientific rationale for the anti-inflammatory therapeutic use of *Cissus quadrangularis* stem. CQE inhibits LPS-induced NO production and iNOS expression in macrophages. These inhibitory effects, at least in part, might be mediated via induction of HO-1 by CQE, thereby leading to decrease in the nuclear level of NF-kB and subsequently the reduction in iNOS expression. Therefore, CQE exerts an anti-inflammatory effect and may contain compounds useful in treating inflammatory diseases including hemorrhoids [19]. In Thailand, the stem of *C. quadrangularis L*. is used traditionally in the treatment of hemorrhoid and irregular menstruation [20]. Plants containing phytoestrogen and triterpenoids have been used in traditional system of medicine for the treatment of osteoporosis. *Cissus quandrangularis L*. (Vitaceae). Thick quadrangular fleshy stem, is an edible plant found in hotter parts of India, Malaya, and West Africa. Commonly known as the "bone setter" the plant ability to join bones. The plant has been documented in Ayurveda for its medicinal uses in gout syphilis and etc. The stem juice is used to treat scurvy and irregular menstruation, the root is used specifically for bone fracture [21]. A number of studies [22-26] have provided additional evidence of the ability of Cissus extracts to stimulate bone growth and healing, and prevention and reversal of osteoporosis. The purpose of this study was to analyze the effectiveness of *Cissus quandrangularis* stem ethanolic (CQE) extract towards its potency as an antibacterial agent against the standard and antibiotic-resistant clinical isolates plus its anti-oxidant and in vitro anti-cancer abilities.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Healthy *Cissus quandrangularis* Stem were collected from the Gandhi market, Tiruchirappalli, Tamil Nadu, India, during August 2016. The stem were washed thoroughly with a few drops of Tepol in running tap water to remove surface debris and then rinsed with distilled water. Further, they were sliced and dried in shade for 2-4 days. The dried rhizomes were ground into fine powder using a mixer grinder.

### 2.2 Extraction procedure

Dried *Cissus quandrangularis* (25g) stem powder was extracted with 400 ml of 95% ethanol (Fisher Scientific, Hampton, New Hampshire, United States) using the soxhalet apparatus for 48 hours. The solvent was evaporated under reduced pressure at 45°C using a rotary evaporator (Buchi R-210, Flawil, Switzerland) to give a yield of 8.4 % of dry extract. The dried *Cissus quandrangularis* stem ethanolic (CQE) extract obtained was stored at -20 ºC until further use.A stock solution of the extract was dissolved in DMSO.Then the stock extract was reconstituted with Dimethyl sulfoxide (DMSO) to produce the desired concentrations for further analysis.

**2.3 Bacterial Strains**

The antibacterial activity was tested against *Staphylococcus aureus* (ATCC 6538)*, Bacillus subtilis, Vibrio cholera, Vibrio parahaemolyticus, Pseudomonas aeruginosa* (ATCC 15442)*, Entero toxigenic Excherichia coli* (*ETEC*) (Resistant to Ampicillin, Chloramphenicol, Gentamicin, Sulfamethoxazole, and Tetracycline) *and Salmonella typhi.* The above standard andclinically isolated strains were obtained from the Department of Biomedical Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. Cultures and morphological features of the strains were confirmed by biochemical characterization.

**2.4 Antibacterial activity**

All the chemicals used for media preparation and the antibiotics were purchased from Hi-Media Limited, Mumbai, India.

**2.4.1 Disc diffusion method**

The test organism was swabbed to the freshly prepared sterile Muller-Hinton agar plates. Sterile paper discs (6mm) were impregnated with various concentrations of the CQE extracts (5-25 mg) suspended in DMSO and DMSOwas used as negative control. The discs were dried to remove the DMSO solvent in a laminar air flow and were placed on the surface of the petriplates using sterile forceps. Plates were incubated at 37˚C for 24hours. Finally the zone of inhibition were noted and tabulated.

**2.5 Free radical scavenging activity**

All the chemicals used for free radical scavenging activity were purchased from Hi-Media Limited, Mumbai, India.

**2.5.1 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) radical scavenging assay:**

$ABTS^{\cdot+}$ scavenging activity of CQE extract was assayed following the method of Re et al., 1999. Seven mM $ABTS^{\cdot+}$ solution and 2.4 mM potassium persulfate solution was prepare as stock solution. The working solution was prepared by mixing equal quantities of each stock solution and allowing them to react in dark for 12 h at room temperature. The diluted solution by mixing 1 ml $ABTS^{\cdot+}$ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm. Fresh $ABTS^{\cdot+}$ solution was prepared for each assay. Plant extract (1 ml) of various concentrations ranging from 20 to 100 mg were allowed to react with 1 ml of the $ABTS^{\cdot+}$ solution and the absorbance was taken at 734 nm after 7 min using UV Vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, United States). The $ABTS^{\cdot+}$ scavenging capacity of the extract was compared with that of butylatedhydroxyltoluene(BHT) standard and percentage of inhibition was calculated using the below formula;

$$ABTS^{\cdot+} \text{ radical scavenging activity (\%)} = [(Abs\ control - Abs\ sample)]/(Abs\ control)] \times 100.$$

Where Abs control is the absorbance of ABTS radical + methanol; Abs sample is absorbance of

ABTS radical + sample extract/standard. The sample concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph of inhibition percentage against sample concentration. All determinations were carried out in triplicates. The ABTS activity radical-scavenging of BHT was assayed for comparison as the positive control.

**2.5.2 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay:**

The effect of extract on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi, 2005. A solution of 0.135 mMDPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of extract in ethanol containing 20 to 100 mg of the extract. The mixtures was vortexes thoroughly and left in dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm using a UV Vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, United States). BHT was used as a reference standard. The ability to scavenge DPPH radical was calculated by the following formula.

*DPPH radical scavenging activity (%) = [(Abs control - Abs sample)]/ (Abs control)] × 100*

Where Abs control is absorbance of DPPH radical + methanol; Abs sample is absorbance of DPPH radical + sample extract/standard. All determinations were carried out in triplicates.

**2.6 MTT *invitro* anti-cancer assay:**

MCF-7 cell line in a monolayer containing approximately $1 \times 10^4$cells were added to each well of a 96 well plate containing Dulbecco's modified Eagle's medium and were incubated for 8h at 37°C in a $CO_2$ incubator (Galaxy® 170 S, Eppendorf, Hamburg,Germany) with a humidified atmosphere of 95% air and 5% $CO_2$. After 8h incubation, cells were exposed to increasing concentrations of CQE extract (2–50 µg/ml) and were incubated for 24 and 48 hours as above. After incubation 20 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well then the cultures were further incubated for 4 h, MTT was aspirated and then 200 ml of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using microplate reader (Bio-rad, USA).

**3. RESULTS AND DISCUSSION**

**3.1 Antibacterial activity**

The results of the antibacterial activity of ethanol extract on the various bacterial strains are as shown in table 1. The table indicated that the extracts showed slight variable degree of antibacterial activity on the different pathogens. The CQE extract showed greatest inhibition on pathogen *Pseudomonas aeruginosa, Salmonella typhi* with a zone of inhibition of 21 mm, in a concentration of 250µg/ml. All the other organisms namely *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera, ETEC* and exhibited a little less inhibition of 18 or 19 mm in the same concentration. The least inhibition was observed on *Staphylococcus aureus* and *Vibrio parahaemolyticus* with a inhibition zone of 6 mm at a concentration of 50 µg/ml.

**Table1**. Zone of inhibition (mm) formed on various bacteria by different concentrations of CQE extract in disc diffusion method

| CQE extract Concentration(µg/ml) | Zone of inhibition of various organisms (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| **50** | 6 | 7 | 7 | 6 | 7 | 7 | 8 |
| **100** | 11 | 10 | 9 | 10 | 11 | 10 | 11 |
| **150** | 14 | 13 | 12 | 13 | 14 | 13 | 15 |
| **200** | 16 | 16 | 15 | 16 | 17 | 15 | 18 |
| **250** | 19 | 19 | 19 | 18 | 21 | 18 | 21 |

(A: *Staphylococcus aureus*, B: *Bacillus subtilis*, C: *Vibrio cholera*, D: *Vibrio parahaemolyticus,* E: *Pseudomonas aeruginosa,* F:*ETEC* and G*: Salmonella typhi*)

### 3.2 Free radical scavenging activity

#### 3.2.1 ABTS radical scavenging activity

The principle behind the technique involves the reaction between ABTS and potassium per sulphate to produce the ABTS radical cation, a blue green chromogen. The presence of antioxidant-reductant, the colored radical is converted back to colorless ABTS, the absorbance of which is measured at 734 nm. The $IC_{50}$ value of the CQE extract and BHT to scavenge the ABTS radical is approximately 30 µg/ml and 35µg/ml respectively, where BHT is the reference standard (Figure 1).

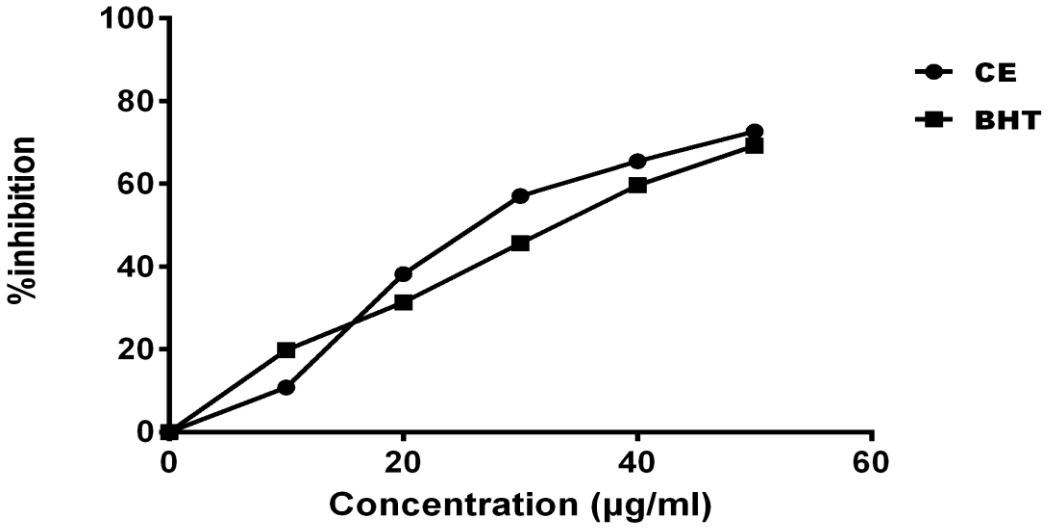


**Figure 1**. ABTS free radical scavenging assay of CQE Extract with butylatedhydroxyltoluene (BHT) control.


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### 3.2.2 DPPH radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The degree of discoloration indicated the scavenging potential of the antioxidants in the extract. $IC_{50}$ value of the CQE extract and BHT to scavenge the DPPH radical is less than 10 µg/ml and 10 µg/ml respectively, where BHT is the reference standard (Figure 2).

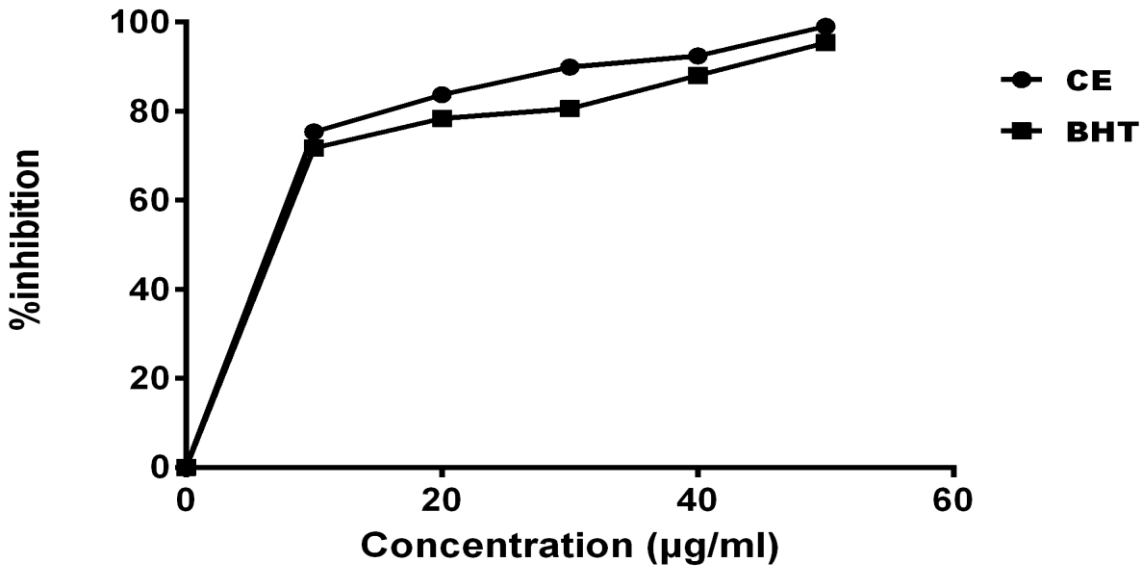


**Figure 2**. DPPH free radical scavenging assay of CQE extract with butylatedhydroxyltoluene BHT) control.

### 3.3 MTT *in vitro* anti-cancer assay

MTT assay was performed to determine the cytotoxic effect of CQE extract on MCF-7 cells. The CQE extract effectively inhibited proliferation of MCF-7 cells in a dose-dependent manner over 24 h (Figure3). The $IC_{50}$value of the CQE extract was 40µg/ml. DMSO was used as control and did not have any anti-proliferation effect on the MCF-7 cell line.

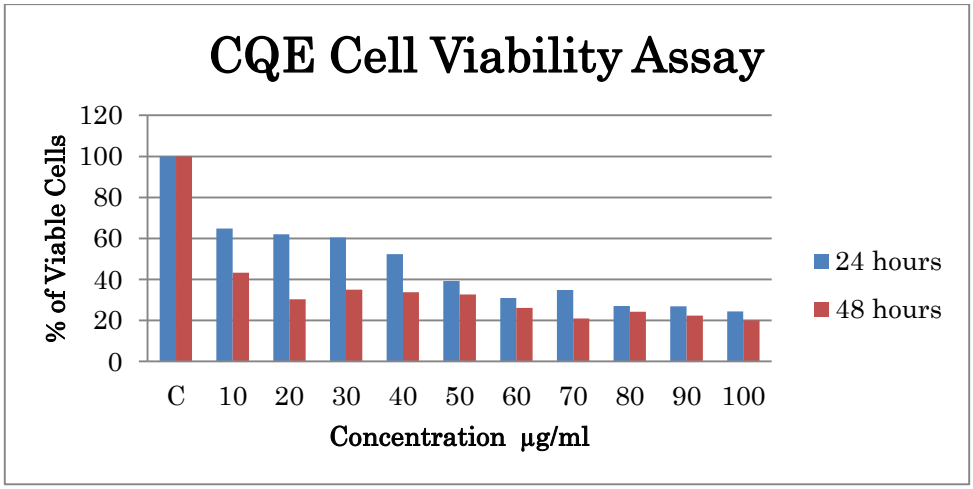


**Figure 3**. MTT assay of CQE extract on MCF-7 cell line shown for 24 and 48 hours.

Most of the pathogens are resistant to present antibiotics so the need of new antibiotics is important in the society. Plants have been a most valuable source for natural product to maintain human health and act as a very good antimicrobial activity. According to world health organization medicinal plants have produce a good antimicrobial and antiseptic compounds. Almost most developing countries 60 to 90% of the people used medicinal plants in their daily dietary [27]. In the present study, totally one plants and 7 spices are used to determine the antibacterial activity. Traditionally, water was used as a good solvent for extraction of compounds from plants and seeds but a few decades, inorganic solvents are used for better solubility of active compounds. *Cissus quadrangularis Linn*. has been widely used as traditional medicine in Africa and Asia including Thailand for the treatment of hemorrhoid, osteoporosis and scurvy [28]. Quercetin and resveratrol, isolated from *C. Quadrangularis* [29], are Major natural antioxidants [30, 31]. In this study, we used ethanol as solvent for extraction of compounds from *Cissus quandragularis* and compare its activity against several human bacterial pathogens. There are many investigators in the past who suggested that plant extracts of different solvents to have great potential of antibacterial activity. In our study, the ethanolic extract of *Cissus quandragularis* showed great anti-bacterial activity against human bacterial pathogens such as *Staphylococcus aureus, Bacillus subtilis, Vibrio cholera, Vibrio parahaemolyticus, Pseudomonas aeruginosa, ETEC* and *Salmonella typhi*. The *Cissus quandragularis* extract demonstrated adequate to strong antibacterial activity, and showed stronger inhibitory effects against all studied human pathogens ($p < 0.05$). The results infered that the most sensitive bacteria were the standard strains namely of *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera, ETEC*. In the current study the ethanolic extract MIC values of Cissus has shown acceptable activity against common human pathogens such as *Vibrio cholera, Vibrio parahaemolyticus, ETEC* and *Salmonella typhi* providing a new lead. *Cissus quadrangularis Linn* (Vitaceae), is widely used in herbal medicine. The stem of *Cissus quadrangularis* has been used for the treatment of irregular menstruation, asthma and hemorrhoids and healing bone fractures [32]. Several studies have demonstrated that the extracts from *Cissus quadrangularis* have anti-oxidant, anti-bacterial, anti-osteoporotic, gastroprotective, analgesic and anti-inflammatory properties [33].This is the reason for the interest towards the inclusion of nontoxic antioxidant flavanoids and polyphenols in the human diet which are abundant in *Cissus quandragularis*. Apart from the studied antiradical activity, CQE ethanolic extract was also subjected to *in vitro* toxicology to ascertain its effectiveness against breast cancer cell line MCF-7. Phytochemical studies of *C. quadrangularis* found several phytochemical constituents such asflavonoids, triterpenoids [34], stilbene derivatives and many others, e.g. resveratrol, piceatannol, pallidolperthenocissin [35], and phytosterols [36]. Pharmacological studies revealed the bone fracture healing property and antiosteoporotic effect of this plant [37]. The antibacterial and antioxidant activities identified of the extract from *C.*


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2018 May – June RJLBPCS 4(3) Page No.101

*quadrangularis* [38]. The clinical trial of *C. quadrangularis* in hemorrhoid patients and found that two capsules of 500 mg dry powder taken twice daily were very effective in the treatment of hemorrhoidal pain and inflammation as well as reducing the size of haemorrhoids [39].

## 4. CONCLUSION

*Cissus quadrangularis L*., a succulent vine from Asia and Africa, has been known for its therapeutic uses since ancient times. The plant is used in the treatment of osteoporosis, asthma, cough, hemorrhoids, and gonorrhea [40]. The results of the present study indicated that the ethanol extract of Cissus rhizome is potent in inhibiting the proliferation of ER-positive breast cancer cells (MCF-7). The results of the current study demonstrate that cissus not only has a powerful inhibitory act on the proliferation of ER-positive (MCF-7) breast cancer cells but also contains significant antibacterial and antioxidant activity. The findings holds promise for further *in vitro* and *in vivo* molecular target-oriented studies to examine the chemoprotective efficacy of ethanol extract of *cissus quandragularis*, particularly for ER-negative breast cancers, which have a poorer prognosis and shorter survival [41].

## 5.ACKNOWLEDGEMENT

No others Acknowledge.

## 6. CONFLICT OF INTEREST

There is no conflict of interest.

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