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Evaluation of Phytochemical Screening and *In vitro* Anti-Oxidant Activities of *Trichosanthes cucumerina*

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Abstract | **Original Research Article**

Trichosanthes cucumerina, a well-known plant, commonly called snake gourd, viper gourd, snake tomato, or long tomato in many countries belongs to the family Cucurbitaceae, is commonly grown in Asian countries including Sri Lanka, India, Malaysia, Peninsula, and the Philippines, and is consumed as a vegetable. The present study screened for phytochemicals present in the plant, and evaluated the in vitro antioxidant activity of the plant using the FRAP and DPPH assay. The phytochemical result for the phenol contents revealed a statistical ($p < 0.05$) difference in the various extracts (10 and 80 mg/ml) when compared to the reference gallic acid. However, the concentration of phenol at 20 and 40 mg/ml chloroform extract was non-statistically ($p>0.05$) different when compared to the reference gallic acid. The result from the flavonoid content showed a statistical ($p < 0.05$) difference across all concentrations of the extracts when compared to the reference gallic acid. The phytochemical results showed that methanol extracts had better yields of 6.149 and 9.286 mg/ml for TPC and TFC respectively when compared to other extracts. The FRAP and PPH results showed a statistical ($p < 0.05$) difference across all extracts when compared to the reference gallic acid. However, for the various tests, methanol extract proved to be more potent, as it had the highest inhibitions of 80.790 % at 40 mg/ml for FRAP and 98.473% for DPPH scavenging activity. The presence of these flavonoid and phenol compounds, as well as antioxidative parameters in the plant, depicts the ability of the plant to prevent free radicals which can cause damage or death of cells. The results support the utilization of the plant for pharmacologic and therapeutic purposes and encourage further characterization study.

Keywords: *Trichosanthes cucumerina*, phytochemical, antioxidant, natural product.

INTRODUCTION

Society has embraced modernization as a lifestyle, which has brought about lots of modifications in society's way of life. These changes, in turn, give birth to the rapid rise of several diseases and disorders. According to previous studies, there have been reports that consuming a good diet, comprising fruits and vegetables, is necessary if the risk factors of many diseases are to be reduced (Rao *et al.*, 2018). Aromatic and medicinal plants (AMPs) are considered an inexhaustible source of bioactive natural substances with different chemical structures and plenty of biological properties, which makes them very coveted by the food, nutraceutical, pharmaceutical and cosmetic industries (Mechqoq *et al.*, 2022).

It is known that medicinal plants have been accepted by both the human and animal systems. The World Health Organisation reported that for primary health care needs, about 80% population in developing countries rely on medicines which are traditionally plant-based. This is a result of the biological potentials of plants which are not limited to antioxidant, antibacterial, antifungal and antiviral activities (Aarti *et al.*, 2020). According to Haziz *et al.*, (2021), the use of plant-based therapy is not only associated with the low economic resources of the populations but also the ineffectiveness of some synthetic drugs. Phytochemicals are non-nutritive plant chemicals that have protective or disease- preventive properties. They are non-essential nutrients. That is, the human body does not require them to sustain life. They are only produced by plants to protect themselves, however, recent research demonstrates that they can also protect against diseases (Raimi *et al.*, 2020). Some phytochemicals present in plants are tannins, terpenoids, alkaloids and flavonoids (Seshadri *et al.*,

2020). Furthermore, several bioactive molecules which serve as a starting material for the synthesis of drugs can be isolated from plants (Abeysinghe *et al.*, 2021; Pathak *et al.*, 2020).

Oxygen metabolism gives rise to superoxide (O_2 ¨), hydroxyl (HO˙) and peroxide radicals (ROO˙), termed Reactive oxygen species (ROS), which when produced in excess can cause severe damage to cells and tissues, resulting in loss of cellular function, oxidative stress, and ultimately, apoptosis or necrosis. Put together, these damages cause many health issues like cancer, respiratory, neurodegenerative, digestive diseases (Borel *et al.*, 2022), autoimmune disorders (Hadera brhane *et al.*, 2018), ageing, inflammation (Shrivastava *et al.*, 2021), heart disease and carcinogenesis. In order to counteract the biomolecules against a ROS attack, antioxidants are needed to neutralise the excessive free radicals, protect the cells against their toxic effects and prevent diseases such as arthritis, stroke, chronic bronchitis (Lukitaningsih *et al*., 2020; Nor *et al.*, 2020), cancer, heart disease, diabetes, Alzheimer's disease, ageing, and cataracts (Lukitaningsih *et al*., 2020). Antioxidants are biologically synthesized as defensive mechanisms having an important role in preventing or alleviating chronic diseases by reducing the oxidative damage to cellular components caused by free radicals (Sajani and Maya, 2020). They stop oxidation chain reactions by making stable, free radicals, and precluding oxidation with others by oxidizing themselves (Ruslin *et al.*, 2021). Antioxidants could be synthetic (Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), propyl gallate), and natural (derived from plants) (Rohman *et al*., 2020; Kokila *et al*., 2020), with the natural antioxidants been explored alternative sources of antioxidants (Lukitaningsih *et al.*, 2020).

Trichosanthes cucumerina, a well-known plant, commonly called snake gourd, viper gourd, snake tomato, or long tomato in many countries belongs to the family Cucurbitaceae, is commonly grown in Asian countries including Sri Lanka, India, Malaysia, Peninsula, and the Philippines, and is consumed as a vegetable (Ruvini *et al.*, 2016). The plant is endowed with arrays of chemical constituents like flavonoids, carotenoids, and phenolic acids which gives it its pharmacological and therapeutic potential, making it prominent in alternative systems of medicine like Ayurveda and Siddha (Devi, 2017). Traditionally, this plant has been used for relieving headaches, alopecia, fever, abdominal tumours, bilious, boils, acute colic, diarrhoea, haematuria and skin allergy. It is also used as a vermifuge, abortifacient, haem agglutinant, refrigerant, laxative, purgative, emetic, bronchitis, cathartic and anthelmintic (Kavitha, 2020). *Trichosanthes cucumerina* houses vital minerals alongside Flavonoids, carotenoids, phenolic acids, and soluble and insoluble dietary fibres. It further contains proteins, fats, fibre, carbs, and vitamins A and E. Its most significant mineral elements are potassium and phosphorus (Amitesh *et al.*, 2022). According to reports, even though the plant is employed in alternative systems of medicine given the secondary metabolites in its possession, the cultivation of *Trichosanthes cucumerina* has stopped, and it is rarely found in home gardens, and as such, the plant is fast going into extinction (Okonwu and Muonekwu, 2019).

Synthetic antioxidants have been and continue to be used as preservative agents to reduce economic losses and protect consumer health. However, there has been a serious concern about the use of synthetic preservatives in the food production chain due to their adverse effects on consumers (Mazhangara *et al.*, 2020). To overcome the drawback associated with the use of synthetic drugs, patients are going back to the use of natural products for their primary health care (Mogole *et al.*, 2020). Plant-based medicines are more easily acceptable to the human body than synthetic drugs. Hence, it is very important to utilize these natural medicines for providing good healthcare service to rural places (Seshadri *et al.*, 2020). Moreover, natural products have been reported to be safer and more effective compared to pharmaceutical drugs, although not many scientific or medical evaluations have been done to evaluate their efficacy (Mogole *et al.*, 2020). This study screened for phytochemicals and determined the *in vitro* antioxidant activities of *Trichosanthes cucumerina* with the intention of discovering the most effective agent for disease management and an effective potential source of natural antioxidants that may help in preventing various oxidative stresses.

MATERIALS AND METHODS

Chemicals and Reagents

All the reagents used were of analytical standard.

Sample Collection

Fresh leaves of *Trichosanthes cucumerina* were collected from a bush in Awo-omamma village of Oru West LGA in Imo state and was authenticated by a Botanist in the department of environmental microbiology, Federal Polytechnic Nekede, Owerri, Imo state.

Sample Preparation

The leaves were washed thoroughly and air-dried until completely dried. They were then ground to fine powder using a laboratory grinding mill and stored in an airtight container until use.

Sample Extraction

100g each of the fine powdered leaves of the plant (*Trichosanthes cucumerina*) was extracted with 500ml methanol, chloroform, N-hexane, and gallic acid, using maceration method for three (3) days, following constant agitation at intervals of time. The extracts were

filtered and evaporated using a rotary evaporator to separate the solvent from residue. The semi-solid residues obtained were stored in sample bottles for further analysis.

Phytochemical Screening

Determination of Total Phenol Content

The number of total phenols in the sample tissues was estimated by the method proposed by Mallick and Singh (1980). The sample (0.5g) was homogenized in 10ml volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The sampling was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipette out and the volume in each tube was made up to 3.0ml with distilled water. Folin- Ciocalteau reagent (0.5ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650nm in a spectrophotometer (Genesys 10-S, USA) against a reagent blank. Standard catechol solutions (0.2 - 1ml) corresponding to 2.0 - 10µg concentrations were also treated as above. The concentration of phenols is expressed as mg/g tissue.

Determination of Total Flavonoid Content

The method proposed by Cameron *et al.*, (1943) was used to sample and estimate flavonoids. The sample (0.5g) was first soaked with methanol: water mixture (2:1) and secondly with the same mixture in a ratio of 1:1. The sample was shaken well and was allowed to stand overnight. The supernatant was pooled and the volume was measured. This supernatant was concentrated and then used for the assay.

A known volume of the sample was pipetted out and evaporated to dryness. Vanillin reagent (4.0ml) was added and the tube was heated in a boiling water bath for 15 minutes. Varying concentrations of the standard were also treated in the same manner.

The optical density was read in a spectrophotometer (Genesys 10-S, USA) at 340nm. A standard curve was constructed and the concentration of flavonoids was calculated thus. The values of flavonoids were expressed as mg/g sample.

Antioxidant Activities

DPPH Spectrophotometric Assay

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the method of Mensor *et al*., (2001). The leaf samples (20µl) were added to 0.5ml of 0.1mM methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the leaf samples, served as the positive control while butylated hydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = \frac{100\text{-A518 (sample) - A518 (blank)} \times 100}{\text{A518 (blank)}}$$

Ferric Reducing Antioxidant Property

The reducing property of the extracts will be determined as described by Pulido *et al.*, (2000). 0.25 ml of the extracts was mixed with 0.25 ml of 200 mM Sodium phosphate buffer of pH 6.6 and 0.25 ml of 1% Potassium ferrocyanide. The mixture was then incubated at 50^{o}C for 20 min, thereafter 0.25 ml of 10% trichloroacetic acid was added and centrifuged at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride and the absorbance was measured at 700 nm.

RESULTS

Phytochemical Screening of *Trichosanthes cucumerina*

Table 1: Total Phenolic content (TPC) of *Trichosanthes cucumerina*

Concentrations	Phytochemical concentrations (mg/ml)			
	Methanol	Chloroform	n-Hexane	Gallic acid
10 mg/ml	2.581^{b}	2.742^{c}	2.601^{d}	-
20 mg/ml	3.750^{c}	3.710^{b}	2.903^{a}	3.690^{b}
40 mg/ml	4.819^{b}	5.423^{c}	3.609^{a}	5.423^{c}
80 mg/ml	6.149^{c}	6.008^{b}	2.964^{a}	7.440^{d}

Across rows, values with different superscript letters are statistically different ($p < 0.05$), while values with the same superscripts are non-statistically different ($p>0.05$).

Table 2: Total flavonoid content (TFC) of *Trichosanthes cucumerina*

Concentrations	Phytochemical concentrations (mg/ml)			
	Methanol	Chloroform	n-Hexane	Gallic acid
10 mg/ml	6.986^{c}	4.638^{b}	4.357^{a}	13.277^{d}
20 mg/ml	8.582^{c}	6.939^{b}	5.671^{a}	15.577^{d}
40 mg/ml	9.286^{c}	4.357^{a}	6.563^{b}	18.160^{d}
80 mg/ml	7.455^{c}	4.779^{b}	4.638^{a}	18.911^{d}

Across rows, values with different superscript letters are statistically different ($p < 0.05$), while values with the same superscripts are non-statistically different ($p>0.05$).

Anti-Oxidant Activities of *Trichosanthes Cucumerina*

Table 3: FRAP Activity of *Trichosanthes Cucumerina*

Concentrations	Percentage inhibition (%)			
	Methanol	Chloroform	n-Hexane	Gallic acid
10 mg/ml	68.685^{c}	50.265^{a}	64.738^{b}	78.422^{d}
20 mg/ml	75.001^{c}	54.212^{a}	67.106^{b}	81.316^{d}
40 mg/ml	80.790^{c}	68.685^{a}	70.790^{b}	84.737^{d}
80 mg/ml	79.737^{c}	69.738^{b}	68.685^{a}	-

Across rows, values with different superscript letters are statistically different ($p < 0.05$), while values with the same superscripts are non-statistically different ($p>0.05$).

Table 4: DPPH Radical scavenging activity of *Trichosanthes cucumerina*

Concentrations	Percentage inhibition (%)			
	Methanol	Chloroform	n-Hexane	Gallic acid
10 mg/ml	93.944^{c}	93.418^{b}	89.573^{a}	98.99^{d}
20 mg/ml	95.945^{c}	93.997^{b}	92.364^{a}	98.99^{d}
40 mg/ml	93.839^{b}	92.154^{a}	94.787^{c}	98.99^{d}
80 mg/ml	98.473^{c}	91.311^{a}	95.945^{b}	98.99^{d}

Across rows, values with different superscript letters are statistically different ($p < 0.05$), while values with the same superscripts are non-statistically different ($p>0.05$).

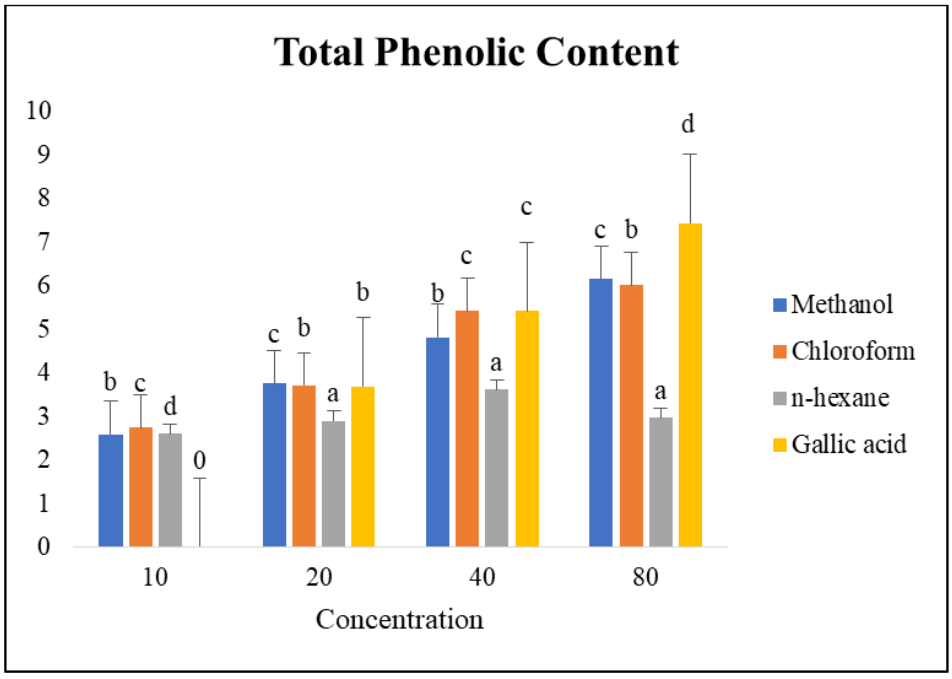

Fig. 1: Values with different superscript letters are statistically different ($p < 0.05$)

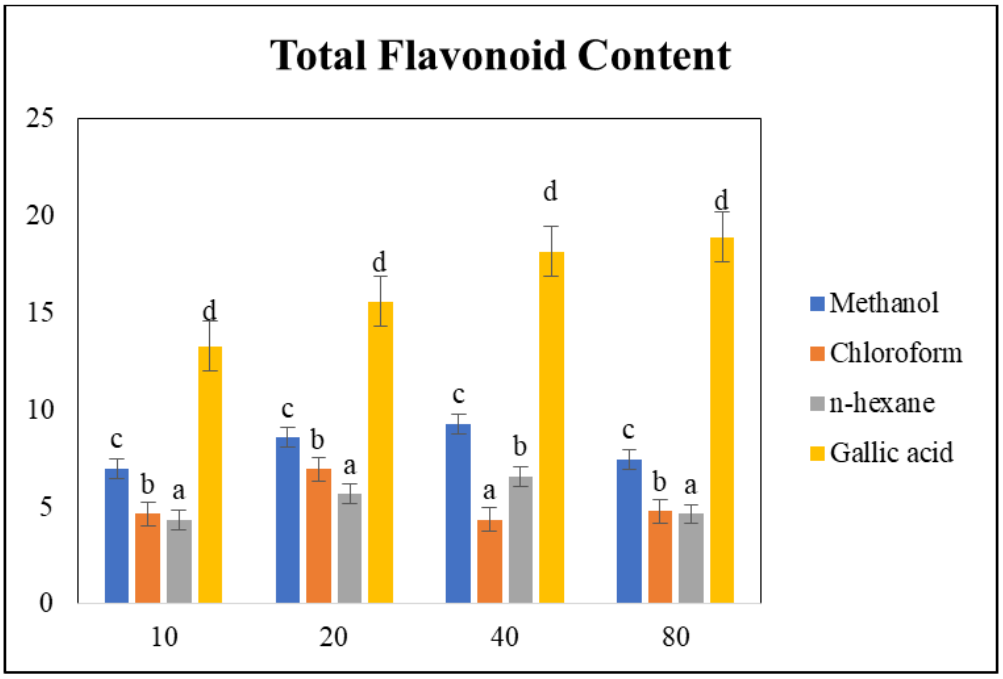

Fig. 2: Values with different superscript letters are statistically different (p < 0.05)

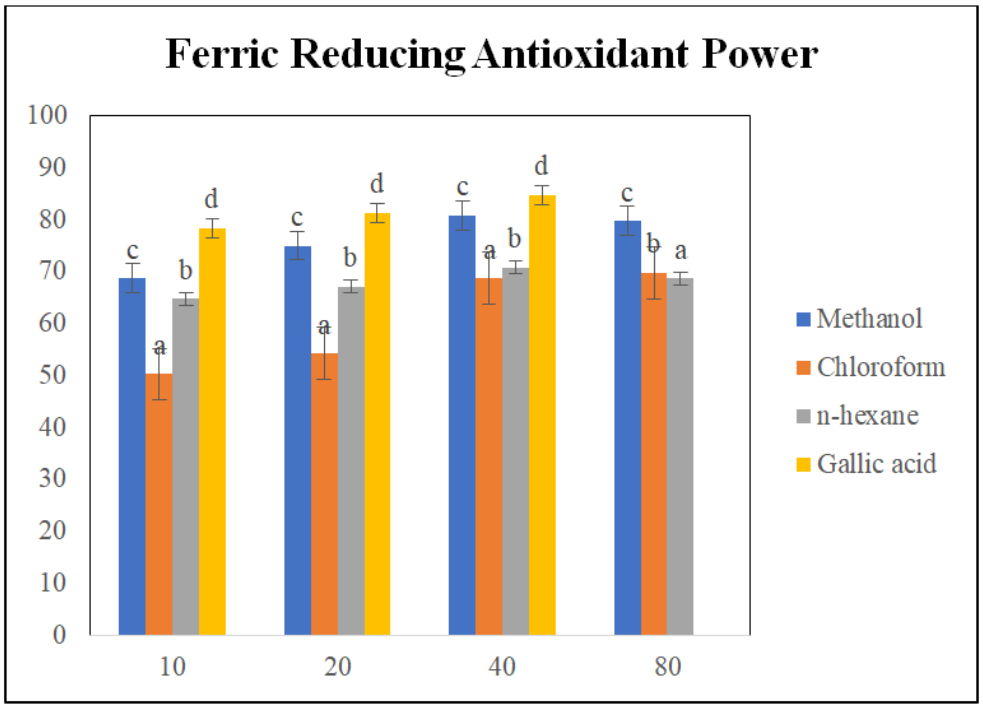

Fig. 3: Values with different superscript letters are statistically different (p < 0.05)

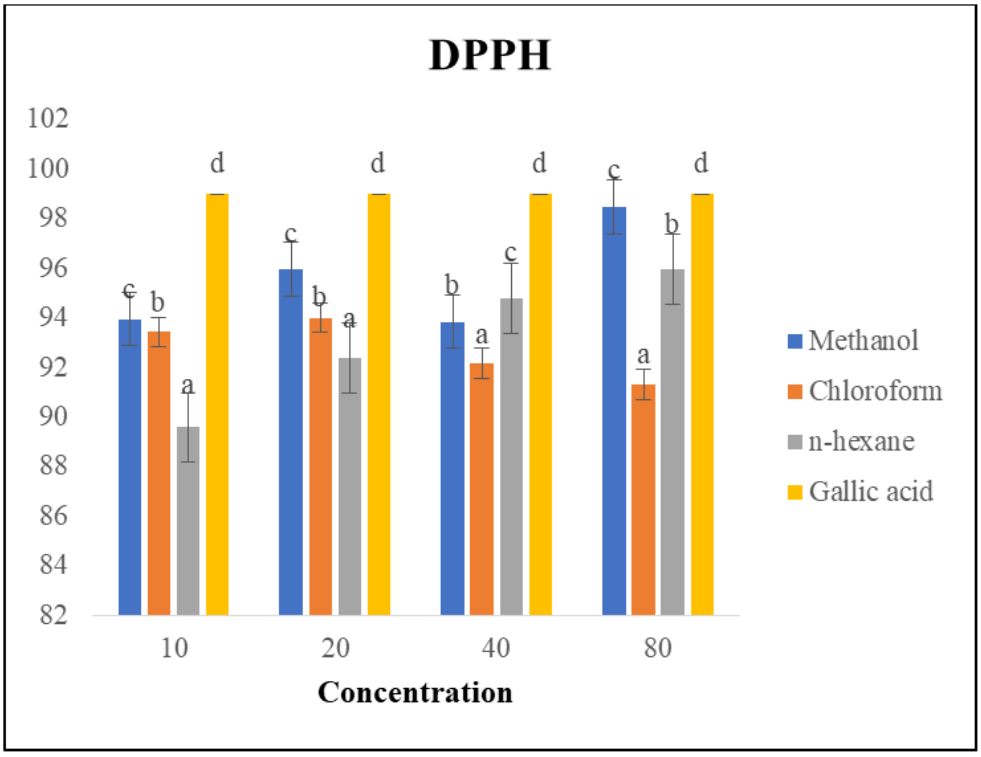

Fig. 4: Values with different superscript letters are statistically different (p < 0.05)

DISCUSSION

Trochosanthes cucumerina was evaluated for the presence of phenolic and flavonoid compounds, as well as its *in vitro* antioxidant activities using FRAP and DPPH assay. The result from Table 1 revealed there was a statistical ($p < 0.05$) difference in the phenol levels of the various extracts (10 and 80 mg/ml) when compared to the reference gallic acid. However, the concentration of phenol at 20 and 40 mg/ml chloroform extract was non-statistically ($p>0.05$) different when compared to the reference gallic acid. The result showed various phenol concentrations of the extracts thus; the methanol extract had TPC of 2.581, 3.750, 4.819, and 6.149 mg/ml; the chloroform extract contained phenol contents of 2.742, 3.710, 5.423 and 6.008 mg/ml; the n-hexane extract had TPC values of 2.601, 2.903, 3.609, and 2.964 mg/ml for the various concentrations of 10, 20, 40, and 80 mg/ml respectively. From the result, methanol had the highest yield of 6.149 mg/ml when compared to the other extracts. This showed that phenolic compounds are found in *Trichosanthes cucumerina*. Phenolic compounds are widely distributed in various higher plant organs such as vegetables, fruits, spices, grains, legumes, and nuts, and play important roles in diverse physiological processes such as plant quality, colouring, flavour, and stress resistance. They are a natural antioxidant, antimicrobial, anticarcinogenic, and anti- inflammatory agents (Zhang *et al.*, 2022), and are an essential part of the human diet (Kumar *et al.*, 2014). Their presence in the plant under study is an indication of the ability to utilize the plant for pharmacologic and therapeutic purposes.

Table 2 records the result of the total flavonoid content (TFC). The result showed a statistical ($p < 0.05$) difference across all concentrations of the extracts when compared to the reference gallic acid. Methanol has the following yield 6.986, 8.582, 9.286, and 7.455 mg/ml; chloroform yielded 4.638, 6.939, 4.357, and 4.779 mg/ml; n- hexane gave 4.357, 5.671, 6.563, and 4.638 mg/ml, while the reference gallic acid had yields of 13.277, 15.577, 18.160, and 18.911 mg/ml for the various concentrations of 10, 20, 40, and 80 mg/ml respectively. Compared to other extracts, methanol had the highest yield of 9.286 mg/ml. The results validate the presence of flavonoid compounds in *Trichosanthes cucumerina*. As a dietary component, flavonoids have high *in vivo* and *in vitro* antioxidant abilities and are thought to have health-promoting properties. They are able to induce human protective enzyme systems. A number of studies have suggested the protective effects of flavonoids against many infectious, and degenerative diseases such as bacterial and viral diseases, as well as cardiovascular diseases, cancers, and other age-related diseases (Kumar and Pandey, 2013). The phytochemical findings from this study justify the study of Palanisamy *et al.*, (2014) who used ethanol extract from the plant to reveal the presence of phenol and flavonoid compounds.

The antioxidant result showed the appreciable ability of the plant to exert FRAP and DPPH potency. The FRAP result as captured in Table 3 showed a statistical ($p < 0.05$) difference across all extracts when compared to the reference gallic acid. The percentage

inhibitions of the various test extracts for 10, 20, 40, and 80 mg/ml respectively are methanol; 68.685, 75.001, 80.790, and 79.737 mg/ml, chloroform; 50.265, 54.212, 68.685, and 69.738 mg/ml, n-hexane; 64.738, 67.106, 70.790, and 68.685 %. The methanol extract exhibited significant radical scavenging activity of 80.790 % at 40 mg/ml. The DPPH result showed a statistical ($p < 0.05$) difference across all extracts when compared to the reference gallic acid. The extracts had percentage inhibition thus; methanol; 93.944, 95.945, 93.839, and 98.473 %, chloroform; 93.418, 93.997, 92.154, and 91.311 %, n-hexane; 89.573, 92.364, 94.787, and 95.945 % respectively.

According to Arawwawala *et al.*, (2011), reactive oxidants produced in biological systems, either by normal metabolic pathways or as a consequence of exposure to external agents have been associated with many different disease conditions. Antioxidants serve as defence factors against free radicals in the body. Enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are the main endogenous antioxidants that oppose oxidative damage; however, this study has demonstrated that the plant *Trichosanthes cucumerina* can be used as an antioxidant agent. The antioxidant potential of the plant can be attributed to the presence of phenolic and flavonoid compounds present in it. The phenolics and flavonoids are characterized by their potential to act as antioxidants. Flavonoids are oxidized by radicals resulting in a more stable, less reactive radical. It appears that the flavonoids stabilize the reactive oxygen species (ROS) by reacting with the reactive compound of the radical (Sudha *et al.*, 2018).

CONCLUSION

This study however can justify the use of *Trichosanthes cucumerina* in traditional medicine practice as a therapeutic agent and can explain its traditional use. The findings of this study support the view that some medicinal plants are promising sources of potent antioxidants and may be efficient as preventive agents for some diseases, thus, supporting the existing comprehensive data on the antioxidant activities of plant material. However, results obtained in the present investigation are promising enough for further isolation and characterization to reveal any novel metabolite of pharmaceutical importance.

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