DELVING INTO PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT POTENTIAL OF ETHYLACETATE LEAF EXTRACT OF GOSSYPIUM HIRSUTUM LINN – A RESEARCH ARTICLE

Dr. Brijesh Kumar Nagwanshi[1], Dr. Arun Kumar[2], Dr Surabhi Verma[3], Dr. Nishant Saxena[4], Prof. Amitabh Pande[5], Neelu Mishra[6] Ashok Kumar Yadav[7] Prabhat Bhagat[8] & Vishwanath Pradeep B [9]

[1]Scientific Officer,State Forensic Science Laboratory, Raipur, Chhattisgarh, India
[2]Lead Research,Target Olympic Podium Scheme,Sports Authority of India,
Ministry of Youth Affairs and Sports, New Delhi
[3]Research Associate, Anthropological Survey of India, Western Regional Centre, Udaipur 313001.
[4]Scientist C, ICMR - National Institute of Research in Tribal Health, Jabalpur, India
ORCID identifier is 0000-0002-3930-4437
[5]Director, Indira Gandhi Rashtriya Manav Sangrahalaya, Bhopal
[6]Assistant Professor ,Faculty of Medical Lab Science Directorate of Training and Technical Education NCT of Delhi Government of Delhi
[7]Research Scholar,Department of Anthropology,Dr. Harisingh Gour University, Sagar, Madhya Pradesh, India
[8] Scientific Officer,State Forensic Science Laboratory, Raipur, Chhattisgarh, India
[9]Research Collaborator, C/ o Innovative association ,Telangana- 502032,India

Corresponding Author: Dr. Arun Kumar, (Lead Research), Target Olympic Podium Scheme
Sports Authority of India, Ministry of Youth Affairs and Sports, New Delhi

KEYWORDS

Gossypium hirsutum, phytochemicals, antioxidants.

ABSTRACT

Gossypium hirsutum is an important plant with therapeutic properties; hence this study seeks to screen the leaf extract for its chemical composition and antioxidant activities to find possible natural sources of novel phytochemicals in pharmaceutical formulations. The qualitative and quantitative phytochemicals screening was conducted using standard procedure, the chemical composition was determined using gas chromatography mass spectrophotometer (GC/MS) while the antioxidant activities was determined using three assays, DPPH, ABTS and NOS. The qualitative phytochemical screening revealed the presence of saponin, alkaloid, phenol, tannin, phytate, steroids, terpenoids, flavonoids and proanthocyanidin. Alkaloid was conspicuously absent in the extract. The qualitative phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, steroids, phytates, terpenoids and proanthocyanidin in different concentrations in which total phenol is the highest 552.64±35.30 mg/100g. In comparison, total saponin is the lowest 22.25±1.41 mg/100g. The GC/MS analysis revealed the presence of 24 compounds which include caryophyllene with the highest percentage composition 33.62%. The antioxidant activities of the extract revealed that the extract has moderate antioxidant activities with EC_{50} of 12.12, 6.01 and 5.01 mg/mL. In conclusion, the leaf extract demonstrated antioxidant properties suitable for exploration in new drug development.

Introduction

Plants since time immemorial have been recognized as a major substance used in food additives, perfumes, cosmetics and in the treatment of various diseases [1]. The need to use plants in the treatment of various diseases have increased due to an increase in antimicrobial resistance.

In recent time, there has been increase in the awareness of phytochemical contents present in plants which are of medicinal importance that can combat micro-organism [2]. India, a country in Asia is blessed with varieties of trees, shrubs and vegetables whose leaves, roots, barks, stems and fruits are of high medicinal potential. In the South West part of India, some shrubs and vegetables are grown around the residential buildings which serve as vegetables for preparing food and as a source of medicine.

Gossypium hirsutum is a common household name in India, it is primarily grown for its cotton fibre commercially but it is planted around private residences for medicinal purposes. It belongs to the family *Malvacea*e and it is believed to be an indigenous effective medicinal plant. The dried root bark of cotton contains gossypol which induces abortion [3]. The leaves of this plant possess anti-malarial properties and it has demonstrated *in-vitro* effects on cell regulation and antitumor activity against mammary carcinoma cell lines [4,5,6].Essential oils in the leaf from G hirsutum have 67.00% hydrocarbon and bet-bisabolol was the major oxygenated component, 13.70%[7] The leafs of *G. hirsutum* have historically been used to treat malaria, hypertension as well as a number of bacteria, fungal and viral illness but the bioactive substances responsible for the physiological effects of this folk medicine are yet to be exhaustively determined. The standardization of this herb is yet to be ascertained and this has resulted into low acceptability of its usage, therefore this study was conducted to obtain, identify and quantify the bioactive compounds in the leaves of *G. hirsutum* and also to determine the antioxidant activities of the ethyl acetate leaf extract of the plant that is responsible for some of its medicinal capabilities.

Materials and Methods

Collection of Plant Samples

Fresh leaves of *G. hirsutum* was collected in India in the month of October, 2022. The sample was collected directly by the researchers, identified and authenticated at the Forestry Research Institute of India, (Osmania University , Hyderabad)

Processing of Plant Sample

The leaf sample was washed with distilled water, air dried for seven days at room temperature and ground into powder using an electric blender. This was kept in a polyethene bag at 4^{o}C for further analysis.

Extraction of the Crude Extract

Exactly 1000g of the pulverized leaf sample was steeped in ethyl acetate for 24 hours, filtered through Whatman no. 1 filter paper and concentrated using a rotary evaporator at 25^{o}C [8]. The extract was stored in firmly kept tight bottles and thereafter kept in a refrigerator at 40^{o}C until the time of analysis.

Percentage Yield of the Leaf Extract

Exactly 1000 g of the powdered leaves were used for the extraction, the percentage yield is expressed using the equation below.

$$\% \text{ Yield} = \frac{Wtofextract}{Wtofsample} \times 100$$

Qualitative Phytochemical Screening of the Leaf Extracts

- **Test for Saponins**

Foam test: Exactly 5 mL of extract was added to 5 mL of water and then it was heated. Froth appears if saponin is present.

- **Test for Alkaloids**

Hager's test: Exactly 2 drops of Hager's reagent were added to 2 mL of extract. Alkaloids are present when there are yellow precipitates.

- **Test for Phenols**

Precisely 4 ml of each of the extracts was treated with 1 mL of concentrated sulphuric acid and 2 drops of sodium nitrate ($NaNO_3$). Exactly 2 mL of sodium hydroxide was added to each of the mixtures. The formation of blue precipitate indicated the presence of phenol. (Iwu*et al.*, 2018)

- **Test for Tannins**

Exactly 2 mL of the extracts was added to 2 mL water and 2-3 drops 5% $FeCl_3$ was added. The presence of tannin was shown by the green precipitate.

- **Test for Phytates**

Phytic acid assay kit by Neogen was used. Exactly 0.5 mL of acetic acid and 2 drops of Neogen kit was added to 1ml of the extract, a blue precipitate appears if phytate is present.

- **Test for Steroids**

Exactly 2 mL of the extract was added to 2 mL $CHCl_3$ and 2 mL concentrated H_2SO_4. Steroids are present, as indicated by the reddish-brown ring at the junction.

- **Test for Terpenoids**

Exactly 5 mL of the extract was mixed with to 2 mL of chloroform, 3mL of H_2SO_4 (concentrated) was carefully added to form a layer. Terpenoids are present when the colour changed to deep red at the surface of the layer formed.

- **Test for Proanthocyanidins**

Proanthocyanidins are rendered visible by staining the extract with freshly prepared 1% vanillin-6-M HCl solution. A distinct red colour develops, if the extract contains proanthocyanidin. (Engstrom *et al*., 2014).

- **Test for Flavonoids**

Precisely 1 mL of extract was added to 1 mL of 10% Pb $C_2H_3O_2$. Flavonoids are present if yellow colour develops.

Quantitative Phytochemical Screening

- **Quantification of Total Phenolic Content**

The phenolic content of the extracts was examined via a spectrophotometric method [20]. Exactly 1 mg/mL of the extract was mixed with the solvent in a test-tube and the mixture was left for about 4 minutes, then 10mL of 7% Na_2CO_3 solution and 13 mL of deinoized distilled water was added, 1 mL of solvent was added to 1 mL of Folin-Ciocalteau reagent to the above mixture. The tube was vortex-mixed for about 25 seconds and kept in the dark at 25^0C for colour development, after which the absorbance was read at 750 nm. The analysis was carried out in triplicate and the results were expressed as mg of gallic acid equivalent/100g of gallic acid using a prepared calibration curve with linear equation as presented below.
Y = 0.009x + 0.012 (R2 = 0.999)\
Here x is the concentration and y is the Gallic acid equipment

- **Quantification of Total Flavonoid Content**

The method of [19] was employed to determine the total flavonoids content. Exactly 0.5mL of 2% $AlCl_3$ in ethanol solution was mixed with 0.5mL of the extract and kept at room temperature for 1h. Absorbance was measured at 420 nm and the flavonoids content was expressed as mg rutin equivalent/100g of rutin using the equation below.

$Y = 0.023 + 0.022$ ($R^2 = 0.982$)

Here x is the concentration and y is the Rutin equivalent

- **Quantification of Total Tannin Content**

Tannin determination was evaluated according to the method described [18] with minor alteration [9] using tannic acid as a standard. 250 mg of the extract was added to 50 mL of distilled water in a conical flask. The mixture was agitated for 1 h using a mechanical shaker, filtered into a 50-mLvolumetric flask and made up to the final volume by the addition of distilled water. An aliquot (1 mL) of the filtrate was mixed with 4 mL of distilled water and treated with 2 mL (10-fold dilution) of 0.1M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The resultant solution was mixed thoroughly and allowed to stay for 10 min; the absorbance was measured at 605 nm against the blank. The quantification was carried out based on the 7-point standard calibration curve of tannic acid (20, 40, 60, 80, 100,140, 200 mg/L) in distilled water. The tannin content was expressed as tannic acid equivalent in mg per 100 g of dry material.

- **Quantification of Total Phytate**

Phytate extraction from plant sources like rice bran typically involves acid extraction, followed by purification techniques like ion exchange or precipitation. [10] The principle of this method relies on conversion of free phytic acid and a colorimetric measurement of the liberated organic phosphorus. Sample (2.0 g) was extracted with 40 ml of 2.4% HCl (68.6 mL of 35% hydrochloric acid in total volume of 1 litre of D_2O) under constant shaking at room temperature (25ºC) for 3 h. The extract was then filtered using Whatman No. 1 filter paper. The content of phytate was determined by using a spectrophotometric method, with an absorbance (A) wavelength at 640 nm, outlined in [11]. The amount of phytic acid was calculated from the organic phosphorus by

assuming that one molecule of phytic acid (containing six molecules of phosphorus (P) was digested [11]. The result was reported in percentage of phytate in 100 gram of sample

- **Quantification of Total Saponin Content**

The Spectrophotometric method of [12] was used for saponin analysis. 1 mL of extract was put into a 250 ml beaker and 100 mL Isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 h to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100 mL beaker and 20 mL of 40% saturated solution of Magnesium carbonate added. The mixture obtained with saturated $MgCO_3$ was again filtered through a Whatman No 1 filter paper to obtain a clear colourless solution. 1 mL of the colourless solution was pipetted into 50 ml volumetric flask and 2 mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 minutes for blood red colour to develop. 0-10 ppm standard saponin solutions (Sigma 47036-50g-F) were prepared from saponin stock solution. The standard solutions were treated similarly with 2 mL of 5% FeCl solution as done for 1 mL sample above. The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectronic 2lD Spectrophotometer at a wavelength of 380 nm. Percentage saponin was calculated using the formula:

$$\text{Saponin (mg/100 mL)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor} \times 100}{\text{Weight of sample x } 10{,}000}$$

- **Quantification of Phytosteroid Content**

To determine the steroid content of a plant sample, you can use methods like the one described by Trease and Evans, involving extraction, washing, and spectrophotometric analysis. A portion of 2 mL was taken from a solution of 2.5 g of powdered plant material prepared in 50 mL of distilled water after vigorous shaking for 1 h. The extract solution was washed with 3 mL of 0.1 M NaOH (pH 9) and later mixed with 2 mL of chloroform and 3 mL of ice-cold acetic anhydride followed by adding two drops of concentrated H_2SO_4 cautiously. The absorbance of both sample and blank were measured spectrophotometrically at 420 nm.

- **Determination of Total Terpenoid Content**

Excatly 1.5 mL Chloroform in each 2 mL microcentrifuge tube was added to 200µL extract. For the standard curve 200 µl Linalool solution in methanol was added to 1.5 mL Chloroform and serial dilution was done[dilution level-100 mg/200 µL to 1mg/200µL (12.965 µM- 1.296 µM) Linalool Conc. In case of serial dilution total volume of 200µl was made up by addition of 95% (v/v) Methanol).The sample mixture was vortex thoroughly and was allowed to stand for 3 min. Exactly 100 µL concentrated Sulfuric acid (H_2SO_4) was added to each 2 mL micro-centrifuge tube. Then the assay tube was incubated at room temperature for 1.5-2 hours in dark. For the standard solution (Linalool) incubation must not be more than 5 minutes and during incubation time the micro-centrifuge tube must not be disturbed. At the end of incubation time a reddish brown precipitation was formed in each assay micro-centrifuge tube. The supernatant reaction mixture was carefully and gently decanted without disturbing the precipitation. The reddish brown precipitation is partially soluble in reaction mixture solution. It was gently decanted, and the supernatant fluid and 1.5 mL of 95% (vol/vol) Methanol were added and vortexed thoroughly until all the precipitation dissolved in Methanol completely. The extract was transferred from the assay tube to Colorimetric cuvette [95% (vol/vol)] Methanol was used as blank] and the absorbance was read at 538 nm. Total Terpenoid Calculation

Standard curve from the blank-corrected at wavelength at 538nm of the Linalool standard was obtained. Total terpenoids concentration of unknown plant sample as Linalool equivalents using the regression equation of Linalool standard curve, was also calculated.

- **Determination of Total Proanthocyanidin Content**

Total proanthocyanidin was determined using the procedure of [14]. The mixture of 3 mL of vanillin - methanol (4% v/v) and 1.5 mL of hydrochloric acid was added to 0.5 mL from 1 mg/mL of aqueous extract and then vortexed. The resulting mixture was allowed to stand for 15 minutes at room temperature followed by the measurement of absorbance at 500 nm. Total proanthocyanidin content was expressed as catechin equivalent (mg/ 100g).

GC-MS Determination of Bioactive Compounds

The GC-MS instrument used was Perkin Elmer Turbo mass spectrophotometer (Norwalk, CT06859, and U.S.A) with XLGC. The column used was Perkin Elmer Elite-5 capillary column, measuring 30m x 0.25mm with a film thickness of 0.25mm consisting of Dimethyl polysiloxane. Helium at a flow rate of 0.5ml/mn was used as a carrier gas, 250^{o}C of inlet temperature was maintained while the oven temperature was programmed to an initial temperature of 110^{o}C for four (4) min after which it was increased to 240^{o}C, and then programmed to increase to 280^{o}C at a rate of 20^{o}C ending with five (5) minutes. The total time used to run per sample was ninety minutes.

***In Vitro* Antioxidant Action**

DPPH Assay

Radical scavenging and antioxidant activity of the extracts of G. *hirsutum* was assessed against free radical DPPH. Six different concentrations (0.0025 – 0.8 mg/mL) of the extracts and synthetic antioxidant vitamin C, gallic acid and E were incubated with DMSO solution of DPPH for about 30 min at room temperature in the dark. The solutions were mixed scrupulously with a vortex machine and the absorbance of each sample was taken at 517 nm. Ethylacetate extract capability to scavenge DPPH free radicals in the solution was calculated using the equation below

$$\text{DPPH Scav activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where Abs control is the absorbance of DPPH + methanol;
Abs sample is the absorbance of DPPH radical + sample extract or standard. The results were expressed in percentage % inhibition = DPPH scavenging activity (%).
Dose – response curve was plotted and IC_{50} value of the synthetic antioxidant, ethylacetate extract was calculated [15].

ABTS Assay:

The modified method of Witayapen [16] was used to evaluate the ABTS activity of the extracts. The working solution was obtained by oxidation of ABTS stock solution (7mM) with 2.4 mM of potassium persulfate in equivalent amounts and themixture was permitted to react for 12 h at 25^{o}C. A portion (1 mL) of theresultant solution was further diluted using 60 mL of methanol to obtainan absorbance of 0.706 ± 0.001 at 734 nm after 7 min using a UV spectrophotometer. Summarily,

five different concentrations (0.025, 0.05,0.1, 0.2 and 0.4 mg mL-1) of each of the extracts were mixed with methanolic solution of ABTS for 7 minutes at 25^{o}C in the dark. The absorbance was then measured spectrophotometrically at 734 nm and the% inhibition of ABTS radical by the extracts and commercial antioxidants (β-carotene and vitamin C) was calculated using the equation described for DPPH assay.

Nitric oxide scavenging assay

A nitric oxide scavenging assay for leaf extracts uses sodium nitroprusside to generate nitric oxide, which is then measured using the Griess reagent, and the scavenging activity is determined by measuring the reduction in nitrite formation. the nitric oxide scavenging activity was carried out. Different quantities of the leaf extracts (0.025, 0.05, 0.1, 0.2 and 0.4 mg mL^{-1}) were combined with 0.5 mL of 10 mM sodium nitroprusside in phosphate-buffered saline, and the mixture was incubated for 150 minutes at room temperature in the dark. Exactly 1mL of water was used as the sample in the control in this experiment. After the incubation period, 0.5 mL of the reaction mixture was added to 1 mL of the sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid). 1 mL of 0.1% naphthyl ethylene diamine dihydrochloride was added after 5 min of incubation, stirred, and incubated for 30 min at 25^{o}C. At 540 nm, the produced chromophore's absorbance was measured. The Trolox standard curve was used to test the extracts' ability to scavenge nitric oxide, and the results were represented as mmole Trolox equivalent (TE) antioxidant capacity per 100 g sample. All analyses were carried out in triplicate.

$$\% \text{ Inhibition } = \frac{\text{Abs control} - \text{ Abs sample}}{\text{Abs control}} \times 100$$

Results and Discussion

Percentage Yield of Crude Extract

The percentage yield of the ethy lacetate leaf extract of *G. hirsutum* is given in Table 1. The yield is 4.49%

Table 1: Percentage Yield of Crude Leaf Extract of *G. hirsutum*

Weight of pulverized sample (g)	Weight of Extract	Percentage Yield
1000	44.90	4.49

Qualitative Phytochemical Screening of Ethylacetate Leaf Extract of *G. hirsutum*

The qualitative phytochemical screening of ethylacetate leaf extract of *G. hirsutum* revealed the presence of phyto compounds such as saponin, phenol, tannin, phytate, steroids, terpenoids and proanthocyanidin as shown in Table 2. This result is in agreement with the work of [18]. These bioactive compounds have been reported to have resistance to microbial pathogens.

Table 2: Qualitative Phytochemical Screening of Leaf Extract of *G. hirsutum*

Phytochemical	Saponn	Alkaloid	Phenol & Tannin	Phytate	Steroids & Terpenoid	Proanthocyanidin	Flavonoid
Presence	+	-	+	+	+	+	+

Quantitative Phytochemical Screening of Ethylacetate Leaf Extract of *G. hirsutum*

The quantitative phytochemical screening of the leaf extract of *G. hirsutum* revealed the presence of phenol flavonoid, tannin, phytate, saponin, steroid, terpenoid and proanthocyanidin in different concentrations (Table 3). The total phenolics is the highest 552.64±35.30 mg/100g, while the saponin content is the lowest 22.25±1.41 mg/100g.

Total Saponin Content

The total saponin content in the extract of *G.hirsutum* is 22.25±1.41 mg/100g as shown in Table 3 and this is in the same range as what was reported by [19]. Saponin are selectively referred to as triterpene glycosides, they are bitter-tasting usually toxic plant-derived organic chemical that have a foaming quality when agitated in water. They decrease blood lipids, lower cancer risk and lower blood glucose [20,21]. A saponin diet can be used in the inhibition of dental caries and platelet aggregation and it is an antidote against acute lead poisoning.

Total Phenolic Content

The total phenolic content in *G. hirsutum* leaf extract is 552.64±35.30 mg/100g (Table 3). [22], reported the absence of phenol in *G. barbadense* but reported its presence in *G. hirsutum*. The result obtained from this study is higher than that reported by [22]and this is a result of the variation in the time of harvest of the leaves and other environmental factors.

Phenols are secondary metabolites synthesized through the Shikimic acid and Phenylpropanoid pathways. They possess numerous bioactive properties and although they are not nutrients but have antioxidant activity [22, 23].

Total Tannin Content

The total tannin content in the *G. hirsutum* leaf extract is 198.44±3.08 mg/100g as shown in Table 3. Tannin is a class of astringent polyphenolic biomolecules that bind to and precipitate proteins and various organic compounds including amino acids and alkaloids. Tannin has antioxidant, antibacterial and anti-inflammatory action in animals [24].

Total Phytate Content

The total phytate content in the leaf extract of *G. hirsutum*is 141.59 ± 6.32 mg/100. it could be observed that this is the first time when phytate will be reported in significant amounts in the leaf extracts in this family and this is at variance with the works of [22, 25]. Phytate is a unique natural substance which could still be found in plants as phytic acid has received considerable attention due to its effects on mineral absorption. Phytic acid prevents the absorption of iron, zinc and calcium and may promote mineral deficiencies and that is why they are referred to as antinutrients, but they have some health benefits. Several factors have been documented to affect reduction in the levels of phytate which include sprouting, fermentation and boiling [26].

Total Steroid Content

The total steroid content in the leaf extract of *G. hirsutum* is 107.90 ± 4.00 mg/100g as shown in Table 3. This value is higher than those reported for the same species in India [27]. Steroids are biologically active organic compounds with four rings arranged in a specific molecular configuration. They are important components of cell membranes which alter membrane fluidity. They act as an anti-inflammatory agent.

Total Terpenoid Content

The total terpenoid content in the leaf extract of *G. hirsutum*is 104.21 ± 2.64 mg/100g as shown in Table 3 and this agrees with the works of [25, 27]. Terpenoids are also known as Isoprenoids, they are a large and diverse class of naturally occurring organic chemicals, which have anticancer, antimicrobial, antifungal and antiviral properties [28, 29].

Total proanthocyanidin content

The total proanthocyanidin in the leaf extract of *G. hirsutum*is 234.50 ± 1.28 mg/100g as shown in Table 3. From the available literature, this is the first time when proanthocyanidin will be reported from the leaves of this plant. This phytochemical is known to protect the heart and cardiovascular system and they work as antioxidants and block nitrosamines from forming. They work with vitamin C to lower the risk of breast cancer and prevent urinary tract infection. [30].

Total Flavonoid Content

The total flavonoid content in the leaf extract of *G. hirsitum*is 312.63± 7.91 mg/100g as shown in Table 3. Flavonoids are secondary metabolites of polyphenols found in plants. They are utilized for the manufacture of pigments that attract insects for pollination in plants. They cannot be synthesized by animals and man [31], but are responsible for taste, colour, and impediment of fat oxidation and prevention of enzymes and vitamin degradation in food [32]. In addition, they also exhibit significant anti-inflammatory, anti-allergic and anticancer activities [33].

The result obtained in the leaf extract in this study is higher than what was reported from Maiduguri, Borno state, Nigeria but the values are in the same range with another work done in southwest, India .

Table 3: Quantitative Phytochemical Screening of the Leaf Extracts of *G. hirsutum*

Extracts	Phenol mg/100g	Flavonoid mg/100g	Tanin mg/100g	Phytate mg/100g	Saponin mg/100g	Steroid mg/100g	Terpinoid mg/100g	Proanthocyanidin mg/100g
GH_{EA}	552.64 ±35.30	312.63 ± 7.91	198.44 ± 3.08	141.59 ± 6.32	22.25 ± 1.41	107.90 ± 4.00	104.21 ± 2.64	234.50 ± 1.28

GC/MS analysis of ethylacetate extract of *G. hirsutum*

The GC/MS analysis of ethylacetate extract of *G. hirsutum*(Table 4) revealed the presence of 24 compounds accounting for 99.98% of the total compound in the extract. The extract consists of 15 major compounds and 9 minor compounds. The major compounds include caryophyllene (33.62%), 3-cyclohexene-1-o1- (1-5-dimethyl-4-hexenyl)-4-methyl (18.43%), palmitic acid (9.26%), α – linolenic acid (7.04%) and Humulene (4.57%). The minor compounds include Bis (2-ethylhexyl) phthalate (0.42%), isomethadone (0.45%) and phytol (0.44%).

β-caryophyllene is a sesquiterpene widely distributed in essential oils, several biological activities are attributed to beta – caryophyllene such as antibiotic, antioxidant, anti-inflammatory, anti-carcinogenic and local anaesthetic activities [34, 35].

Palmitic acid is a saturated fatty acid, it is not synthesized in the human body but from other fatty acid and carbohydrate [36]. Increase in the proportion of Palmitic acod on the body cells has been shown to promote inflammatory process [37].

Humulene, also known as α-caryophyllene is a ring-opened isomer of β-caryophyllene. It possesses powerful anti-inflammatory activity [38], it is an effective analgesic when taken topically or orally [39] and a wound healing activity [40].

Neophytadiene a minor compound in the extract is a diterpene majorly found in the methanol extract of the crataevanurvale plant. It was reported with anxiolytic-like activity, sedative properties and antidepressant – like actions [41]. Phytol which is also one of the minor compounds present in this extract is an acyclic alcohol of diterpene frequently available in certain aromatic

plants' essential oil. Phytol and its derivative have been shown to have antibiotic, anti-inflammatory and anticonvulsant activity [42].

Table 4: Chemical composition of ethylacetate leaf extract of *G. hirsutum*

S/N	R.T	Compound	% composition
1.	1.373	Levopropoxyphene	2.07
2.	1.516	Isomethadone	0.45
3.	2.226	1-(1,3-Benzodioxol-4-yl)-N-methyl propan-2-amine	0.77
4.	2.403	Mephedrone	1.01
5.	2.517	7-Bromoheptanoic acid	3.35
6.	3-988	Buutyloxitol	1.79
7.	4.898	2-Butoxylethyl acetate	0.61
8.	6.099	Copaene	1.16
9.	6.282	Caryophyllene	33.62
10.	6.403	Humulene	4.57
11.	6.546	(Z)-1-1Methyl-4, (6-methylhept-5-en-2-ylidene) cyclohex-1-ene	5.82
12.	6.580	Cadina-1(6),4-diene	0.76
13.	7.061	3-cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	18.43
14.	7.450	Neophtadiene	0.74
15.	7.827	Palmitic acid	9.26
16.	8.131	4-carbamoyl-2-phenyl-2-oxazoline	0.75
17.	8.165	Phytol	0.44
18.	8.279	α-linolenic acid	7.04
19.	8.302	Stearic acid	0.80
20.	9.218	Bis (2-ethylhexyl) phthalate	0.42
21.	10.305	Xylose, 4,5-diacetamido-4,5-dide oxydiethylmercaptal, L-	1.90
22.	10.385	1-Bromo-11-iodoundecane	1.31
23.	11.793	dl-alpha-Tocopherol (Vitamin E)	1.68
24.	13.464	Beta-sitosterol	1.23
		Total	99.98

Note: Minor compound <1%
Major compound > 1%

Antioxidant Activities of the Leaf Extracts of *G. hirsutum*

Antioxidant activities of the different crude extracts of *G. hirsutum* is presented in Table 5. It has been documented that it is preferable to use more than one method when carrying out tests on antioxidant activity [43]. The higher antioxidant activity of the methanol extract of *G. barbadense* is due to higher quantities of phytochemicals found in the extract because antioxidant properties have been reported to be positively correlated with the amount of flavonoids and phenolics in a plant extract [44, 45, 46]. In a previous study, it was reported that *G. hirsutum* has higher phenolics and flavonoids content and therefore higher antioxidant activity, but, in the present study, *G. hirsutum*has higher phenolics and flavonoids content than what was reported in the previous literature therefore it has better antioxidant capacity as shown in Table 5.

Table 5. Antioxidant activities of the leaf extracts of *G. hirsutum*

Extracts	Assay % Scavenging			Effective Concentration @ 50% EC_{50}		
	% inhibition DPPH	Trolox equivalent ABTS	Nitric oxide scavenging	DPPH mg/mL	ABTS mg/mL	NOS mg/mL
GH_{EA}	20.93 ± 0.53	40.45 ± 1.13	50.27 ± 0.28	12.12	6.01	5.01
VIT C	69.85	-	90.26	0.007	-	0.005
Gallic acid	68.00	-	82.01	-	-	0.006
VIT E	16.92	-	7.40	-	-	0.067

Conclusion

The results obtained in this study depicts that *G. hirsutum* leaf extract is rich in phytochemicals with proven antioxidant activities. The phytochemical analysis conducted on the leaf ethyl acetate extract revealed the presence of pharmacologically active phytochemicals. The findings of the present study suggest that the leaves of the investigated plant could be a potential source of natural antioxidants that can have great importance in preventing the progress of aging and age-associated oxidative stress-related degenerative diseases.

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