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# Antioxidant activity and FTIR analysis of *Ageratum conyzoides* L

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**Abstract**
*Ageratum conyzoides* L. belongs to family Asteraceae which consists of approximately 30 species and has broad pharmacological activities but most commonly used for wound healing. The purpose of this study to screening of bioactive components and to calculate the anti-oxidant potential of these weed. The FT-IR spectroscopy is used to identify and characterized the functional groups present in various plant parts with different solvents. The peaks of leaf and flower were resembled the absorption frequency of quercetin in both extracts. The result of this study concealed that flowers of *Ageratum conyzoides* L. possess significant amount of phenolic (270.58±0.705 µg GAE/g DW), flavonoid component (107.69±1.114 µg Quercetin/g DW) and also shown noteworthy antioxidant properties among all plant parts with aqueous and ethanol solvents. The regression analysis shows that flavonoid compounds contribute to about 81% (*r2* = 0.8084, $P < 0.05$) of radical scavenging properties. Thus, it revealed that this weed has a considerable antioxidant activity due to the presence of TFC.

**Keywords:** *Ageratum conyzoides* l., weed, wound healing, FT-IR spectrum analysis

## Introduction
Medicinal plants are the richest bio-resources for traditional medicines, pharmaceuticals industries and their phytochemicals are used to synthesis novel drugs. (Ncube *et al*., 2008) [1]. Phytochemistry is very important and relevant because it deals with the relationship between natural products and organic chemistry which can be directly tested for their pharmacological activity (Temidayo *et al*.,2013) [2]. Plants are rich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, flavanoids, quinones, betalains and other metabolites which are rich in antioxidant activity (Aiyegoro *et al*.,2010) [3]. Herble drugs having free radical scavengers which are used for their therapeutic purpose (Halliwell *et al*.,1994; Hakiman *et al*.,2009) [4, 5]. Natural material is great source of antioxidants which possibly safe, effective and cheap. Therefore, it become the target for number of research studies (Mundhe *et al*., 2011) [6]. *Ageratum conyzoides* L. belongs to family Asteraceae which consists of approximately 30 species. It is commonly called as goat weed due to peculiar odour liked Australian male goats (Kamboj and Saluja.,2008) [7]. In past, this weed used in various diseases such as common wound and the burned one, antimicrobe, arthrosis, headache, and dyspnea. In Africa, goat weed has been applied as a wound dressing agent as well as an antiulcer agent (Kotta *et al*.,2020) [8]. The leaves are used to prevent tetanus as well as consumed as vegetables (Kaur and Dogra, 2014) [9]. The roots are good sourced to treat tumours and diarrhoea in a baby. Likewise, the flower is also used in relieve itching, cough, vermifuge and as tonics (Yadav *et al*., 2019) [10]. It is also reported that, this weed increased a soil nutrient composition in agriculture practises by utilizing as an organic material (Anhar *et al*.,2018) [11].
The extract of goat weed has a hypoglycemic effect (Stéphane and Bernard, 2013) [12]. Aqueous extract of the leaves of *Ageratum conyzoides* L. has been reported to prevent coagulation of blood and the bleeding time was also decreased (Akah, 1988) [13]. Aqueous extract of leaves is combined with honey which was significantly accelerate wound healing process (Mustafa *et al*.,2005; Oladejo *et al*.,2003) [14, 15].
*Ageratum conyzoides* L. contain phytochemicals with various potencies for development of herbal drugs as well as its pharmaceutical formulation (Kotta *et al*.,2020) [8]. In this research article, the active phytoconstituents were screened followed by antioxidant activities and FT- IR analysis.

## Material and Methods
### Collection and extraction of plant material
The whole plant material of *Ageratum conyzoides* L. was collected from Godhra forest division, near Poonam dam, Godhra, Panchmahal, India in November, 2019. The authentication of plant was carried out by Department of Botany, Gujarat University, Ahmedabad. The plant materials (roots, stems, leaves and flowers) of this weed were collected, washed with water and kept for drying at room temperature than homogenized into powder and stored in airtight bottles till further use. Dried plant materials were extracted by using two solvents -aqueous and ethanol in soxhlet extractor (Patel *et al*., 2019) [16].

### Determination of yield percentage (%)
The percentage yield extracts were calculated using formula which was based on dry weight:

$$\text{Yield \%} = \frac{\text{W1 X 100}}{\text{W2}}$$

Where W1= weight of extract after Solvent evaporation; W2 = Weight of the grinded leaf powder

**Phytochemical screening of various extract of *Ageratum conyzoides* L.**

The extracts were subjected to qualitative analysis for various phytochemical constituents according to standard protocol (Harborne, 1998 & 1993, Adebayo & Ishola, 2009) [17, 18].

**FT-IR (Fourier transforms infrared) spectrum analysis**

Identification of different bioactive compounds present in various extract of *Ageratum conyzoides* L, FT-IR spectrum analysis was performed. In FT-IR spectra, few drops of extracts were placed over instrument glass through which laser passes between the range from 400 to 4000 cm-1 and their functional group were identified in accordance to characteristic peaks (Chaudhary *et al*., 2019) [19].

**Determination of total phenolic content**

One ml of various extracts (roots, stems, leaves and flowers) of *Ageratum conyzoides* L. were mixed with 1.5 ml of Folin-Ciocalteu reagent than 4 ml (20% Na2CO3) was added and incubated for 30 minutes at room temperature and measured absorbance at 765 nm against a blank using spectrophotometer. Average of each triplicate were quantified by the standard curve of gallic acid solution (Y = 0.0038X - 0.0026, R2 = 0.9993) (Patel *et al*., 2018) [20].

**Determination total flavonoids content**

Thousand microlitre of various extracts (roots, stems, leaves and flowers) of *Ageratum conyzoides* L. were mixed with 100µl of aluminium chloride (10%) and potassium acetate (1M). This mixture was incubated at room temperature and measured the absorbance at 415 nm against a blank using spectrophotometer. Average of each triplicate were quantified by the standard curve of quercetin solution (Y = 0.0056X + 0.0199, R2 = 0.9963) (Patel *et al*., 2019) [16].

**Antioxidant assay**

**DPPH free radical scavenging activity**

Free radical scavenging activity of different extracts of roots, stem, leaves and flowers of *Ageratum conyzoides* L. were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH), according to the method described by Chang *et al*., 2001 [21]. The equal amount of sample solution was mixed with an equal amount of 0.1mM methanol and ethanol solution of DPPH. The mixture was kept in dark for half an hour. After incubation, the absorbance of the mixture was read against a blank at 517 nm using UV-visible spectrophotometer. The average of each triplicate was taken for determination of percentage inhibition (*I*%) which is calculated as per the following equation: *I* %= A control- A sample /A control x 100, where A control is the absorbance of the control (containing all reagents except the test compound), A sample is the absorbance of the experimental sample with all reagents. The IC50 value was calculated from the plot of inhibition (%) against the concentration of the extract. Here, ascorbic acid was used as standards (Hossain *et al*., 2013) [24]

**Reducing power assay**

The reducing power assay was determined by the method of Oyaizu (1986) [23] with some modification. One ml of different extracts (roots, stems, leaves and flowers) of *Ageratum conyzoides* L. with different concentration were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%). The mixture was incubated in water bath at 50ºC for 15 minutes. After 20 minutes,2.5 ml of trichloro acetic acid (10%) was added and centrifuged at 3000 rpm for 10 minutes. In supernatant solution, 2.5 ml deionized water was added and 0.5 ml freshly prepared ferric chloride solution (0.1%) also added. Then absorbance was measured at 700 nm against blank using UV spectrophotometer. Increased absorbance of the reaction mixture indicates increase in reducing power. This experiment was done three times and average data was noted. Here, ascorbic acid used as standard (Dewan *et al*.,2013) [24].

**Ferric ion reducing antioxidant power assay (FRAP assay)**

The FRAP assay was performed using standard protocol given by Benzie and Strain 1996 with some modification. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 40 mM HCL and 20 mM FeCl3· 6H2O (10:1:1 v/v), warm up at 37 °C in water bath before use. The freshly prepared FRAP reagent was added to different concentration of standard solution of FeSO4.7H2O and the reaction mixture was incubated in dark then measured the absorbance at 593 nm using a spectrophotometer (Svilaas *et al*., 2004). The result was quantified by the standard curve of FeSO4.7H2O having regression formula Y = 0.0025X+0.8043, R2 = 0.9767. The result was expressed as mM Fe (II)/g dry weight of plant material.

**Phosphomolybdenum assay**

The total antioxidant capacity (TAC) of various plant parts (roots, stems, leaves and flowers) of *Ageratum conyzoides* L were determined by the green phosphomolybdenum method of Prieto *et al*., 1999 [27], with different concentration. Then, 0.5 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) was mixed in each aliquot which were incubated in boiling water bath at 95˚C for 90 minutes and measured the absorbance at 695 nm against a blank. The higher absorbance value is indicated higher antioxidant activity (Adetuyi *et al*.,2018) [28].

**Statistical analysis**

The result is expressed as mean ± standard deviation. In all antioxidant assay, observation was analysed using one way analysis of variance (ANOVA). IC50 value calculated using linear regression curve. The correlation between TPC, TFC, and antioxidant activity is presented by Pearson correlation coefficient.

Results are considered statistically significant when *P*-values < 0.05. Spread sheet soft-ware (Microsoft Excel, version 2107) are used for all statistical analysis.

**Result**

Estimation of yield percentage of *Ageratum conyzoides* L.

The total yield percentage of *Ageratum conyzoides* L was calculated by using formula.

In aqueous extract of different plant parts (roots, stems, leaves and flowers) are contained 9.57, 11.94, 19.84 and 20.17 % yield respectively. The leaf confined highest yield which is 25.40 % in ethanolic extract when compared with stem (15.97 %), flower (14.79 %) and root (4.13%) of same extract.

**Phytochemical screening of different extract of *Ageratum conyzoides* L.**

The dried extract of root, stem, leaf, flower of *Ageratum conyzoides* L. are used for qualitative analysis. The result shows that plants having different phytochemicals such as flavonoids, steroids, phenolic compounds, proteins and cardiac glycosides are shown in table 1.

**Table 1:** Qualitative analysis of different parts of *Ageratum conyzoides* L.

| **Phytochemical constituents** | ***Ageratum conyzoides* L.** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Root | | | | Stem | | | Leaf | | | Flower | |
| AE | | EE | | AE | EE | | | AE | EE | | AE | EE |
| Flavonoids | | | | | | | | | | | | |
| Alkaline reagent test | + | | + | + | + | | + | | | + | + | + |
| Lead Acetate test | + | | + | + | | - | + | | | + | + | + |
| Phenolic/Tannin | | | | | | | | | | | | |
| Ferric Chloride test | + | | - | + | | - | + | | | + | + | + |
| Lead Acetate test | + | | + | + | + | | + | | | + | + | + |
| Folin ciocalteu reagent | + | | + | + | + | | + | | | + | + | - |
| Steroids | | | | | | | | | | | | |
| Libermann Burchard's test | - | | - | - | | - | - | | | + | - | + |
| Salkowski test | - | | - | - | | - | - | | | + | - | + |
| Cardiac Glycosides | | | | | | | | | | | | |
| Keller-Killiani test | - | | - | - | | - | - | | | - | - | - |
| Legal's test | + | | - | + | | - | + | | | - | + | - |
| Protein | | | | | | | | | | | | |
| Millon's test | - | | + | - | | + | - | | | + | - | + |
| Biuret test | - | | - | - | | - | - | | | - | - | - |

AE=Aqueous Extract, EE=Ethanolic Extract, + :Present ,- :Absent

**FT-IR (Fourier transforms infrared) spectrum analysis**

Identification of different bioactive compounds present in various extract of *Ageratum conyzoides* L were performed by using fourier transforms infrared (FT-IR) which are shown in tables and figure. C-CO-C stretching and bending vibration of ketones were observed at 1163 cm-1 confirms the absorption spectra of flavonoid quercetin (Thiyagarajan *et al*.,2016). The gallic acid also indicated peaks at 1701 cm-1(Carboxylic acid) ,1615 cm-1(C=C stretching) and 1265 cm-1(C=O stretching) (Lam *et al*.,2012). The FT-IR spectra of different parts of *Ageratum conyzoides* L were compared with the standards gallic acid and quercetin as shown in figure 1 & 2. Gallic acid contained characteristic bands at 3265 cm-1(O-H group), 2653 cm-1 (O-H stretching carboxylic acid) and 1699.7 cm-1 (C=O stretching conjugated acid). The bands at 1613 cm-1(C=C stretching α-β-unsaturated ketone) and 1230 cm-1 (C-O stretching vinyl ether). Quercetin contained bands at 3265.1 (O-H stretching vibration of phenol) and 1736.9 (C=O stretching δ lactone). The band at 1233.7 (C- O-C-O stretching aryl ether) and 1159.2 was assigned to C-CO-C stretching and bending vibration of ketones. The absorption frequencies of leaf and flower were resembled the absorption frequency of quercetin as well as gallic acid in both extract while the absorption frequencies of root and stem were similar to the absorption frequency of gallic acid in both extract (Table 2 and 3).

**Table 2:** The FT-IR spectra of aqueous extract *Ageratum conyzoides* L.

| **Aqueous (Wave number cm-1)** | | | | **Possible functional group** |
|---|---|---|---|---|
| Root | Stem | Leaf | Flower | |
| 3295.0 | 3239.1 | 3328.5 | 3257.7 | Leaf & Flower: O-H stretching alcohol intermolecular bonded and O-H stretching vibration of phenol<br>Root & Stem: O-H stretching alcohol intermolecular bonded |
| 2944.6 | 2970.7 | 2918.5 | --- | Root and Stem: C-H stretching alkene<br>Leaf: O-H stretching carboxylic acid (Centered on 3000 cm-1) |
| --- | --- | 2851.4 | --- | C-H stretching alkane |
| 1744.4 | 1736.9 | 1733.2 | --- | C=O stretching δ lactone |
| 1718.3 | --- | --- | --- | C=O stretching cyclohexanone or C=O stretching carboxylic acid dimer |
| --- | --- | 1625.1 | --- | C=C stretching conjugated alkene |
| 1561.8±5 | 1561.8±5 | 1595.3 | 1561.8±5 | C=C stretching cyclic alkene |
| --- | --- | 1461.1 | --- | C-H bending alkane (methyl group) and O-H bending alcohol |
| 1386.6 | 1375.4 | 1371.7<br>1323.2 | 1394.0±5 | O-H bending Phenol |
| 1215.1 | 1230.0±5 | 1237.5 | --- | Root and Stem: C-O stretching Vinyl ether<br>Leaf: C-O stretching aryl ether |
| 1028.7 | 1028.7 | 1025.0 | 1028.7 | C-O stretching Vinyl ether |
| 980.3±5 | --- | --- | 980.3±5 | C=C bending alkene monosubstituted |
| --- | --- | 868.5 | --- | C-H bending 1,2,4-Trisubstituted |
| 797.7 | 797.7 | 775.3 | 797.7 | C-H bending monosubstituted benzene derivatives Leaf: C-H bending monosubstituted benzene derivatives and<br>C-H bending 1,2,3 trisubstituted |
| 670.9 | 670.9 | --- | 667.2 | C=C bending alkene disubstituted (cis) |

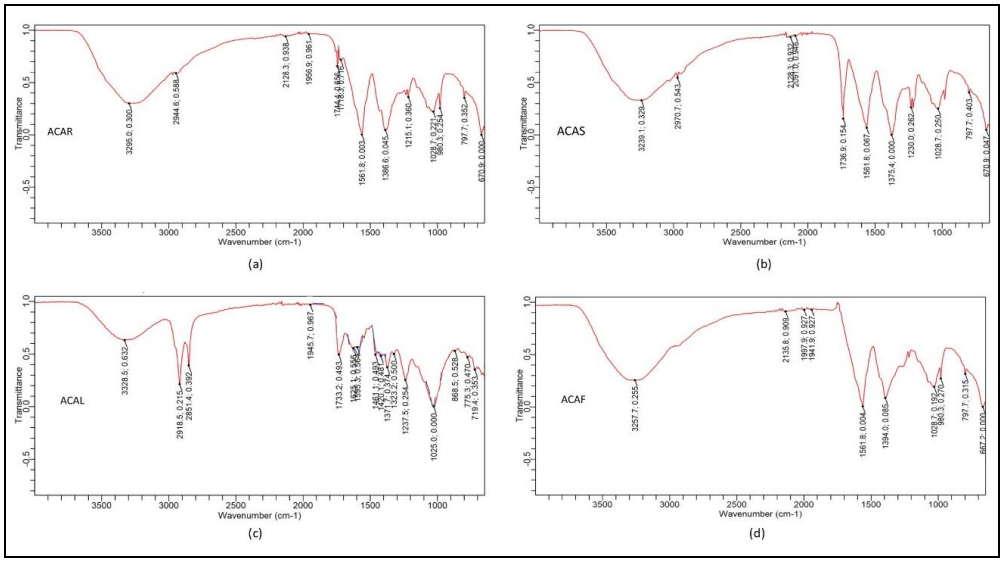


**Fig 1:** The FT-IR spectra of aqueous extract of *Ageratum conyzoides* L. ACAR- *Ageratum conyzoides* aqueous root, ACAS-Stem, ACAL-Leaf, ACAF-Flower

**Table 3:** The FT-IR spectra of ethanolic extract *Ageratum conyzoides* L

| Ethanol (Wave number cm-1) | | | | Possible functional group |
|---|---|---|---|---|
| **Root** | **Stem** | **Leaf** | **Flower** | |
| 3205.5 | 3317.3 | 3346.5 | 3350.9 | leaf & Flower: O-H stretching alcohol intermolecular bonded and O-H stretching vibration of phenol Root & Stem: O-H stretching alcohol intermolecular bonded |
| --- | --- | --- | 3011.7 | Aromatic C-H stretching alkene |
| 2922.2 | 2922.2 | 2922.2 | 2922.2 | O-H stretching carboxylic acid (Centered on 3000 cm-1) |
| --- | 2851.4 | --- | 2855.1 | C-H stretching alkane |
| --- | 1736.9 | --- | 1736.9 | C=O stretching δ lactone |
| --- | --- | --- | 1606.5 | C---C aromatic ring stretch |
| 1587.8 | 1591.6 | 1595.3 | --- | C=C stretching cyclic alkene |
| 1513.3 | --- | --- | 1513.3 | C=O aromatic stretch |
| --- | --- | --- | 1457.4±5 | C-H bending alkane (methyl group) |
| 1386.6 | 1379.1 | 1379.1 | 1371.7 | O-H bending Phenol |
| 1256.1 | 1237.5 | 1237.5 | 1237.5 | C-O stretching alkyl aryl ether |
| 1151.7 | --- | 1159.2 | 1159.2 | C-CO-C stretch and bending in ketone |
| --- | --- | --- | 1118.2 | Unknown |
| 1073.5 | 1025.0 | 1069.7 | 1032.5 | C-O stretching Vinyl ether |
| 1028.7 | | 1036.2 | | |
| --- | --- | 1036.2 | --- | C-O stretching primary alcohol |
| 808.8 | --- | --- | 812.6 | C-H bending 1,3-disubstituted |
| --- | 797.7 | --- | 700.7 | Stem: C-H bending monosubstituted benzene derivatives Flower: C-H bending 1,2,3 trisubstituted |

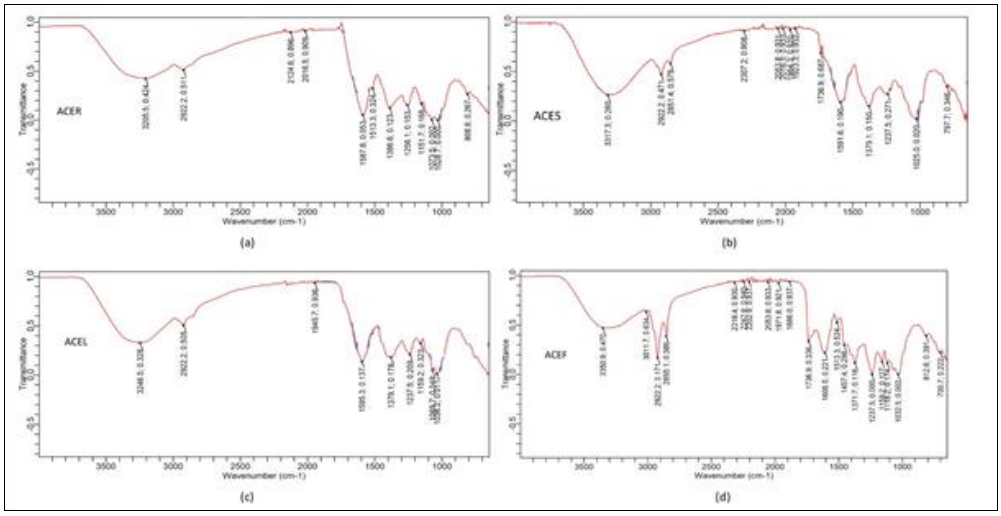


**Fig 2:** The FT-IR spectra of ethanolic extract of *Ageratum conyzoides* L. ACER- *Ageratum conyzoides* aqueous root, ACES-Stem, ACEL-Leaf, ACEF-Flower

**Determination of total phenolic content**

In the present study, the total phenolic content with various plant parts extract varied from 76.33 µg to 270.58 µg of GAE/g of dry extract which are presented in table 4. The flower shows excessive phenolic content (270.58 µg GAE/g) in aqueous extract while the root ensures least phenolic content in same extract (76.33 µg GAE/g) (Figure 3). The notable concentration of phenolic compound found in flower and leaf are 209.67 µg GAE/g and 207.97 µg GAE/g in ethanolic extract respectively.

**Table 4:** Total phenolic content (TPC) and total flavonoid content (TFC) of various plant parts of *Ageratum conyzoides* L.

| Plant parts | TPC (µg GAE/g DW) | | TFC (µg Quercetin /g DW) | |
|---|---|---|---|---|
| | Aqueous | Ethanol | Aqueous | Ethanol |
| Root | 76.33±0.493 | 111.12±1.389 | 42.98±0.544 | 29.46±0.585 |
| Stem | 79.14±0.924 | 129.29±1.359 | 42.21±0.447 | 38.45±1.307 |
| Leaf | 174.83±0.986 | 207.97±1.180 | 59.53±0.269 | 86.03±0.511 |
| Flower | 270.58±0.705 | 209.67±1.510 | 107.69±1.114 | 74.82±0.603 |

The average value of triplicate experiments are represented as mean ± standard deviation.
GAE- Gallic acid equivalent, DW- Dry weight

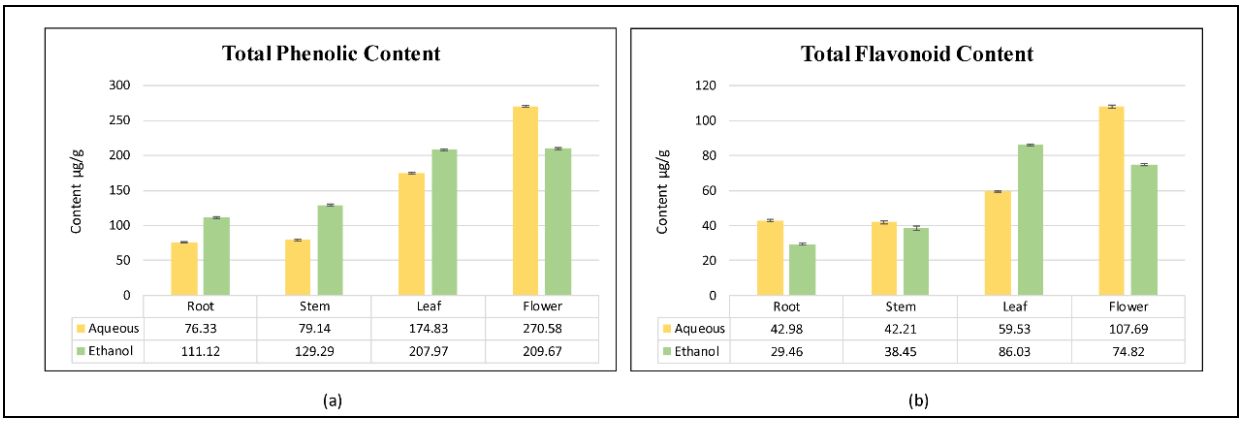


**Fig 3:** Total phenolic content of different extract of *Ageratum conyzoides* L.

**Determination of total flavonoid content**

All the plant parts of *Ageratum conyzoides* L. displayed notable amount of flavonoid which are shown in figure 5. The highest amount of flavonoid present in aqueous extract of flower
(107.69 µg of Quercetin /g) followed by ethanolic extract of leaf (86.03 µg of Quercetin /g). The lowest amount of flavonoids are seen in root (29.46 µg of Quercetin /g) and stem (38.45
µg of Quercetin /g) of ethanolic extract. The flavonoids levels are summarized in table 4.

**DPPH free radical scavenging activity**

Free radical scavenging activity of aqueous and ethanolic extracts of *Ageratum conyzoides* L. found to be increased with increase in the concentration of extract. The percentage inhibition of DPPH and IC50 values for samples with standard are given in table 5 and 6. In the present investigation, the flower of *Ageratum conyzoides* L. is exhibited 84.55 % inhibition at the concentration of 200 µg/ml followed by standard ascorbic acid (88.01%) at same concentration in aqueous extract. The IC50 values of flower and ascorbic acid are found to be 82.39 µg/ml and 110.48 µg/ml respectively. Leaf and root show lowest inhibition 50.79 %,56.67 % at the concentration of 200 µg/ml in aqueous extract. The IC50 values of leaf and root are found to be 188.54 µg/ml and 180.31 µg/ml respectively. The flower and leaf show maximum (90.19 %,83.61 %) free radical scavenging activity with IC50 values 182.78 µg/ml and 177.20 µg/ml at the concentration of 350 µg/ml in ethanolic extract. The % DPPH curves for samples and standard are presented in figure 4.

**Table 5:** DPPH scavenging activity of aqueous extract of various plant parts of *Ageratum conyzoides* L.

| Concentration (µg/ml) | | | % Scavenging | | |
|---|---|---|---|---|---|
| Ascorbic acid | | Root | Stem | Leaf | Flower |
| 25 | 10.97±1.14 | 17.00±0.05*** | 16.41±0.59** | 17.65±0.44*** | 26.14±0.43# |
| 50 | 23.10±1.38 | 23.17±0.54# | 21.77±0.54# | 19.64±0.49* | 40.67±0.34# |
| 100 | 43.09±1.71 | 30.81±0.55*** | 33.26±0.49*** | 32.90±0.51*** | 59.97±0.14# |
| 150 | 72.45±1.30 | 40.90±0.31# | 47.27±0.71# | 44.23±0.14# | 76.39±0.49** |
| 200 | 88.01±1.86 | 56.67±0.58# | 57.65±0.84# | 50.79±0.11# | 84.55±0.69* |
| IC50 Value (µg/ml) | 110.48 | 180.31 | 166.29 | 188.54 | 82.39 |

The average value of triplicate experiments are represented as mean ± standard deviation.
*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, # - Not significant

**Table 6:** DPPH scavenging activity of ethanolic extract of various plant parts of *Ageratum conyzoides* L.

| Concentration (µg/ml) | | | % Scavenging | | |
|---|---|---|---|---|---|
| Ascorbic acid | | Root | Stem | Leaf | Flower |

| | | | | | |
|---|---|---|---|---|---|
| 100 | 47.39±3.00 | 16.03±0.46# | 33.98±0.63** | 37.82±0.64** | 23.94±0.54*** |
| 150 | 52.40±6.69 | 28.10±0.43** | 38.02±0.63* | 45.90±0.41# | 41.28±0.41* |
| 200 | 59.72±5.78 | 34.21±0.54** | 43.28±0.80** | 54.43±0.46# | 56.51±0.69# |
| 250 | 73.79±6.18 | 50.13±0.76** | 50.13±0.46** | 59.62±0.41* | 72.31±0.30# |
| 300 | 79.18±6.66 | 59.85±0.52** | 59.08±0.69** | 67.58±0.92* | 85.00±0.72# |
| 350 | 84.19±6.01 | 77.35±0.46# | 71.54±0.63* | 83.61±0.19# | 90.19±0.72# |
| IC50 Value (µg/ml) | 123.70 | 248.99 | 229.38 | 177.20 | 182.78 |

The average value of triplicate experiments are represented as mean ± standard deviation.
*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, # - Not significant

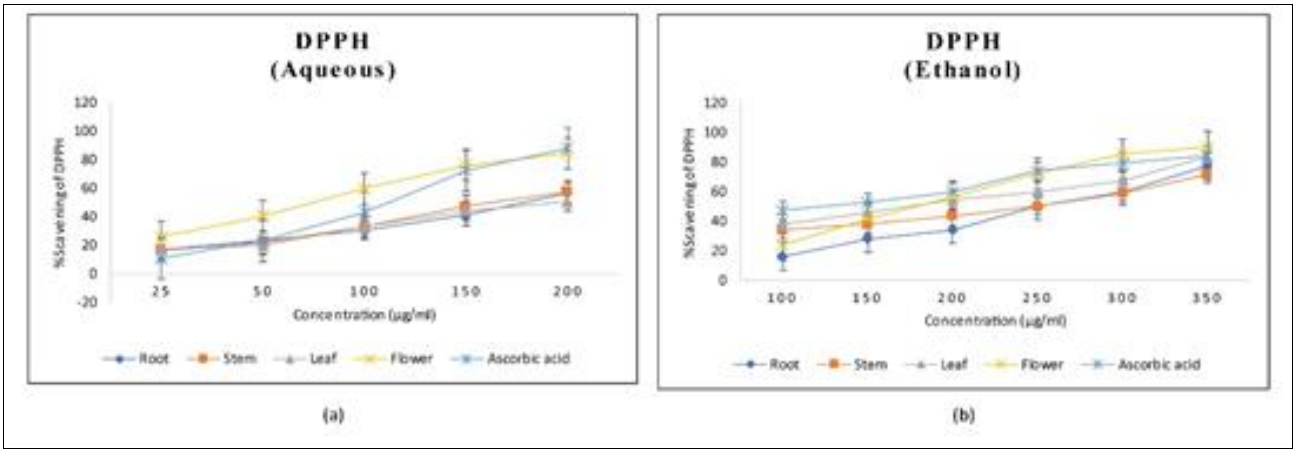


**Fig 4:** DPPH radical-scavenging activity of aqueous and ethanolic extract of various parts of *Ageratum conyzoides* L.

**Reducing power assay**

Ascorbic acid is used as positive control in reducing powers assay. The maximum absorbance for the aqueous extract is found to be 1.293nm, 1.202nm at 500 µg /ml concentration in flower and leaf respectively and at same concentration, the absorbance of standard is 1.024nm at same concentration (Figure 5). The reducing power of *Ageratum conyzoides* L in aqueous extract with standard present in the following order: Flower > Leaf > Ascorbic acid >Stem > Root. The highest absorbance is observed to be 1.741nm,1.469nm at 1000 µg /ml concentration for leaf and flower respectively, followed by ascorbic acid at same concentration which is 1.157nm in ethanolic extract. The reducing power of *Ageratum conyzoides* L in ethanolic extract with standard express in the following order: Leaf > Flower > Ascorbic acid >Stem > Root. The aqueous extract of leaf and flower are showed significant value which is less than 0.05 at 300

µg /ml concentration while the ethanolic extract of leaf and flower are disclosed significant value which is less than 0.01 at 500 µg /ml concentration and 0.001 at 600,900 µg /ml concentration. The other plant parts are not significant in both extracts with different concentration. The reducing properties are generally associated with the presence of reductions, which can break the free radical chain by donating a hydrogen atom (Duh, 1994).

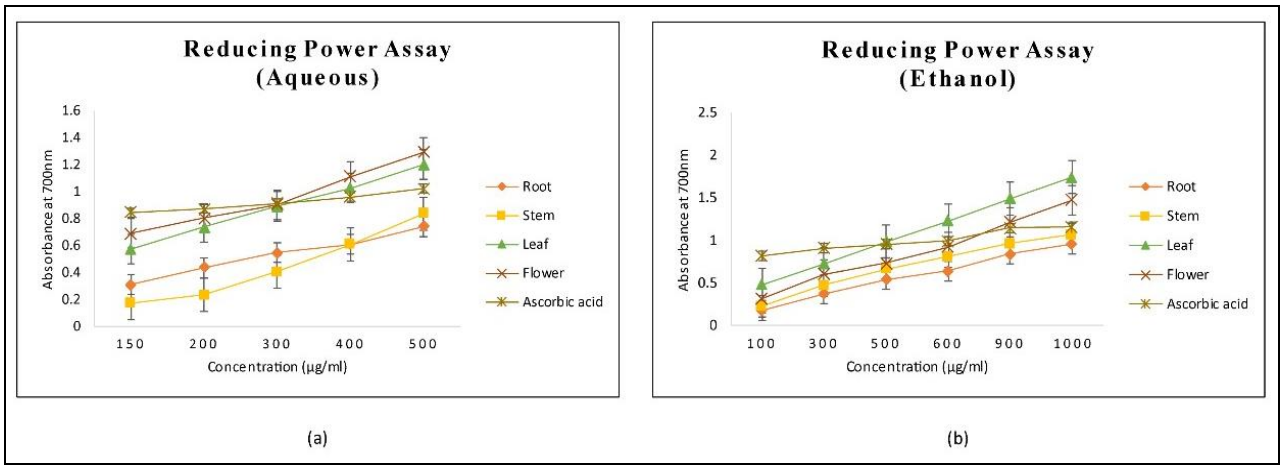


**Fig 5:** Reducing power of aqueous extract of *Ageratum conyzoides* L.

**Ferric ion reducing antioxidant power assay (FRAP assay)**

The FRAP values of aqueous and ethanolic extract of various plant parts of *Ageratum conyzoides* L. are given in table 7. In this assay, FeSO4.7H2O is used as references standard. In ethanolic extract, all plant parts of *Ageratum conyzoides* L. exhibit upper most ferric ion reducing activity which ranges from 420.54 to 579.34 µg of mM Fe (II)/g of dry extract at 500 µg/ml concentration as compare with aqueous extracts. The root shows low ferric ion reducing activity in aqueous extract (95.53 µg of mM Fe (II)/g of dry extract) at 500 µg/ml concentration (Figure 6).

**Table 7:** FRAP value and total antioxidant capacity of different extract of *Ageratum conyzoides* L.

| Plant parts | FRAP (µg mM Fe(II)/g DW) | | Phosphomolybdenum (µg AAE /g DW) | |
|---|---|---|---|---|
| | Aqueous | Ethanol | Aqueous | Ethanol |
| Root | 95.53±6.143 | 420.54±3.629 | 148.66±0.577 | 269.5±3.500 |
| Stem | 127.94±3.642 | 460.14±2.722 | 3.00±2.000 | 177.5±2.291 |
| Leaf | 290.53±4.313 | 465.21±3.780 | 244.33±2.081 | 316.16±2.753 |
| Flower | 340.34±4.241 | 579.34±2.837 | 433.33±3.055 | 323.66±6.601 |

The average value of triplicate experiments are represented as mean ± standard deviation
AAE- Ascorbic acid equivalent, DW- Dry weight

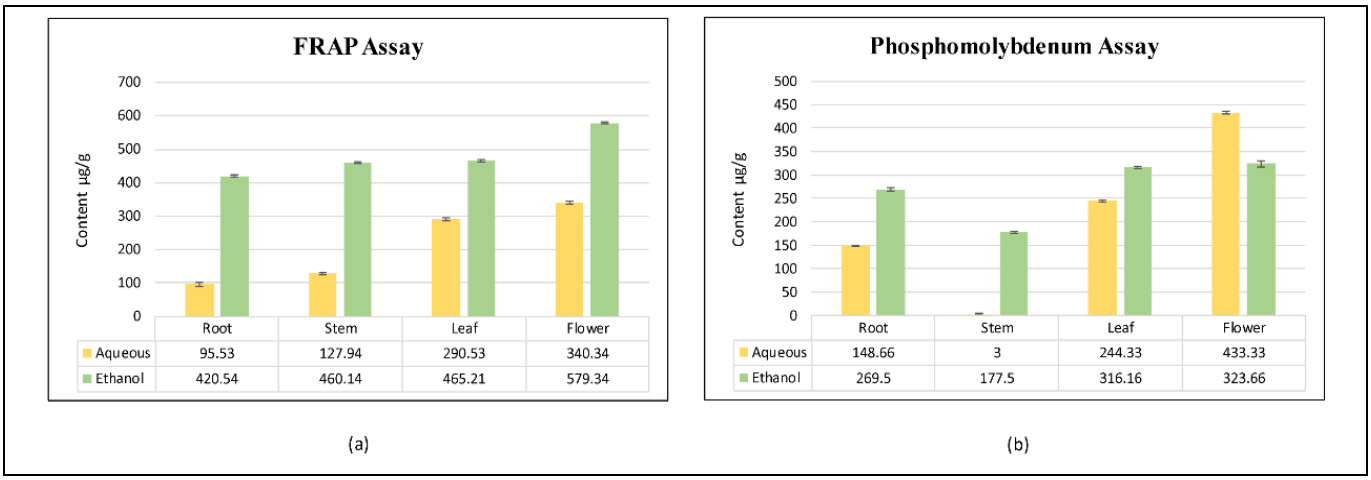


**Fig 6:** FRAP value and total antioxidant capacity of *Ageratum conyzoides* L.

**Phosphomolybdenum assay**

In the present investigation, the total antioxidant capacity with various plant parts extract varied from 3.00 µg to 433.33 µg of AAE/g of dry extract which are presented in table 7. The flower shows high total antioxidant capacity (433.33 µg AAE/g) in aqueous extract (Figure 6).

**Statistical analysis**

The DPPH radical scavenging activity is showed a strong negative correlation with TFC ($r$=−0.909, $P$ < 0.05) in aqueous extract (Table 8). DPPH radical scavenging activity is indicated by a negative correlation since the radical content decreases as activity increases. Therefore, TFC of aqueous extracts may play a major role in the increase in DPPH radical scavenging activity. The DPPH radical scavenging activity is presented a strong negative correlation with TPC ($r$=−0.993, $P$ < 0.01) and TFC ($r$=−0.990, $P$ < 0.01) in ethanolic extract. Therefore, TPC and TFC of ethanolic extracts may play a key role in the increase in DPPH radical scavenging activity. TPC and TFC are disclosed a strong positive correlation to FRAP assay ($r$=0.960) and ($r$=0.860) in aqueous extract of plant. The total antioxidant capacity is reported a strong positive correlation with TPC($r$=0.939417) and TFC($r$=0.92409) in aqueous extract. The relationship between total flavonoid content and antioxidant using DPPH assay is shown in figure 7. The regression analysis shows that flavonoid compounds contribute to about 81% ($r2$ = 0.8084, $P$ < 0.05) of radical scavenging properties.

**Table 8:** Pearson's correlations between antioxidant activities measured using different assays and total phenolic/flavonoid contents (Aqueous and ethanolic extract)

| Aqueous | TPC | TFC | DPPH | FRAP | TAC |
|---|---|---|---|---|---|
| TPC | 1.000 | | | | |
| TFC | 0.967156 | 1.000 | | | |
| DPPH | -0.78168 | -0.90918* | 1.000 | | |
| FRAP | 0.960325 | 0.860512 | -0.60088 | 1.000 | |
| TAC | 0.939417 | 0.92409 | -0.71693 | 0.860417 | 1.000 |
| Ethanol | TPC | TFC | DPPH | FRAP | |
| TPC | 1.000 | | | | |
| TFC | 0.98366 | 1.000 | | | |
| DPPH | -0.99324** | -0.99038** | 1.000 | | |
| FRAP | 0.728093 | 0.594246 | -0.68225 | 1.000 | |
| TAC | 0.739881 | 0.726706 | -0.67444 | 0.472089 | 1.000 |

**$P \leq 0.01$ *$P \leq 0.05$

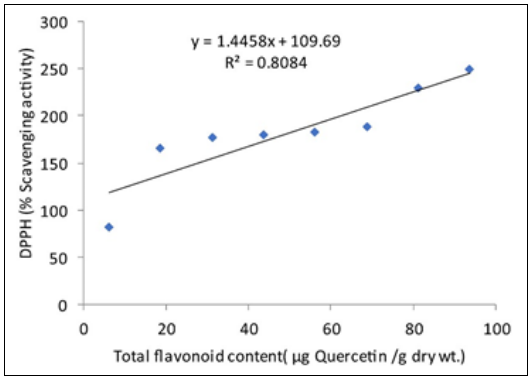


**Fig 7:** Relationship between total flavonoid content and antioxidant activity by DPPH assay

## Discussion

The leaf confined highest total yield in 80 % ethanol as compared to aqueous extract. This weed is rich in polyoxygenated flavonoids, among them 14 are polymethoxylated and polyhydroxy flavones which include quercetin, kaempferol and their glycosides (Okunade, 2002) [32]. The phytochemical substances such as quercetin, kaempferol, glycoside, tannin, and other polyphenolic compounds can reduce inflammation significantly, which is induced by carrageenan and histamine activities in ethanolic extract of Ageratum conyzoides L. (Hassan et al., 2017) [33].

The peaks at 3492 cm-1,3367 cm-1 and 3285 cm-1 correspond to different modes of the OH groups. The bands between 2850 to 2950 cm-1 assigned to stretching vibration of aliphatic CH, CH2and CH3 side chain of aromatic rings (Slawinska et al.,2007) [34]. In aqueous extract, the bands are appearing at 2944.6 cm-1 and 2970.7 cm-1 (OH stretching) which is similar to Eclipta alba (Muruganantham et al., 2009) [35]. In aqueous ethanol extract 2933 cm-1 (C-H stretching), 1122-1cm-1 (C-CO-C stretching) (Ragavendran et al., 2011). The peak at 3332.99 cm revealed the presence the alcohols, phenols (O–H stretch, H–bonded) and the peak at 2970.38 and 2885.51-1 cm refers to the presence of alkanes (C–H stretch). In aqueous extract, the peak at 1759.08-1cm-1 and 1666.50 cm corresponds the carboxylic acid group (C=O stretch) in fruit extract of M. dactyloides (Rajiv et al.,2016). In ethanol extract of Areva lanata L., bands are showed at 2918 cm-1 (C-H stretching), 1654 cm-1, 1718 cm-1 (C=O stretching), 1165 cm-1, 1249 cm-1 (C-CO-C stretching). Eleven major peaks are observed in aqueous extract of C. bicolor 3452.58, 3302.13, 2931.80, 2376.30, 2229.71, 1585.49, 1419.61, 1076.28, 655.80, 617.22 and 428.20 cm-1 in the region between 500-4000 cm-1 (Vanitha et al.,2019) [38]. In aqueous extract of leaf exhibited low phenolic content (174.83 µg GAE/g) and flavonoid content (59.53 µg of Quercetin /g) when compared with polyphenolic content reported by Adetuyi *et al*.,2018 [28] which was 48.9 mg GAE/g and 18.7 mg of Quercetin /g in leaves of same plants. In ethanolic extract of leaf revealed low phenolic content 207.97 µg GAE/g when compared with phenolic content reported by Dewan *et al*.,2013 [24] and Hossain *et al*.,2013 [24] which were 378.37 mg GAE/g and 378.37 mg GAE/g in leaves of same plants respectively. The ethanolic extract of leaves has a strong natural antioxidant effect against the free radical which is used in the treatment of erectile dysfunction induced by oxidative stress Adetuyi *et al*.,2018 [28]. The ethanolic extract of *Ageratum conyzoides* L. has gastroprotective and antiserotonergic properties which could be mediated by its antioxidant activity (Shirwaikar *et al*.,2003) [39]. Phenolic compounds such as flavonoids, phenolic acids, tannins, etc., are very principal constituents for the free radical scavenging activities of plants and this phenolic groups react as hydrogen donors and neutralize the free radicals (Aliev *et al*., 2009; Kulisic *et al*., 2004; Moncada *et al*., 1991; Soare *et al*., 1997; Vinson *et al*., 1995) [40, 41, 42, 43].

The reducing power assay demonstrated that the antioxidant compounds can donate electron which can cause reduction during the process of lipid peroxidation (Tachakittrungrod *et al*.,2007). The ethanolic extract of leaf is recorded high ferric ion reducing ability which is significantly ($P < 0.01$) higher than aqueous extract. The total antioxidant capacity is low in leaf of aqueous extract (174.83 µg AAE/g) as reported by Adetuyi *et al*., 2018 [28] which was 33.5 mg AAE/g in leaves of same plants. The aqueous extract of leaf is exhibited low total antioxidant capacity 174.83 µg AAE/g when compared with leaves of *Eucalyptus globules* which was 23.32 mg AAE/g, reported by Gupta *et al*., 2009[46]. Tannins and flavonoids are commonly found in plants, due to these phytoconstituents *Ageratum conyzoides* L. may be showed the high inhibition value in ethanol extract (Vinson *et al*., 1995) [44]. Therefore, total flavonoid content might be play crucial role in the increase in DPPH free radical activity.

## Conclusion

The present investigation reveals that, the flower of *Ageratum conyzoides* L. shows excessive phenolic content and flavonoid content in aqueous extract. The flower shows highest percentage of free radical scavenging activity in aqueous and ethanolic extract. The reducing power of *Ageratum conyzoides* L in aqueous extract with standard is presented the following order: Flower > Leaf > Ascorbic acid >Stem > Root. Flower shows highest ferric ion reducing activity in aqueous and ethanolic extract. Observation from the present study suggests, that flower of *Ageratum conyzoides* L. contains significant amount of phenol, flavonoid component and also shows significant antioxidant properties among all plant parts with both solvents. The result of FTIR shows the presences of the functional group in all extracts which have medicinal properties and can be used to treat against various disease such as, anti inflammentory, antimicrobial and anticancer agent.

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