Phytochemical evaluation, HPTLC analysis and in-vitro antioxidant activity of hydroalcoholic leaf extract of Grewia hirsuta collected from Western Ghats forest

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Article History:
Received on: 11 Apr 2020
Revised on: 10 May 2020
Accepted on: 13 May 2020

Keywords:
Antioxidant activity, Grewia hirsuta, Hydroalcohol extract, Nagbala

ABSTRACT
In-vitro antioxidant action of hydroalcoholic leaf extract of Grewia hirsuta (HAEGH) has been examined using one, “1-diphenyl-2-picryl-hydrazil (DPPH) free from radical scavenging” actions. The plant collected from the forest of the Western Ghats region of Karnataka province. The motive for plant collection from a specific location is the plant of forests exhibits the variation in growth, quantity, and quality of their active ingredients and secondary metabolites due to influence ecological factors like effect changes in location, soil, climate, etc. The work corresponds to preliminary phytochemical investigation for diverse phytoconstituents and quantitative phytochemical analysis of total phenolic, flavonoids & alkaloids content (TPC, TFC & TAC respectively) was evaluated with advanced methods. “HPTLC (High performance thin-layer) chromatography” fingerprint investigation was achieved for qualitative determination of the likely number of elements present in the hydroalcoholic extract. In-vitro antioxidant activity of HAEGH has been determined through hydroxyl radical scavenging assay that exhibited strong dose-dependent antioxidant activity as compared with standards compound, ascorbic acid. The IC50 value of HAEGH found, 25.90 % inhibition and for ascorbic acid, it was 17.68%. The Preliminary phytochemical estimation found presence of flavonoids, alkaloids, glycosides, phenol, proteins, diterpins and quantitative phytochemical analysis estimation of TPC, TFC & TAC found to be 3.627%, 4.059% & 5.671% respectively. HPTLC analysis of HAEGH at 354nm reveals the presence of a compound with Rf value 0.44 compare with Rf value 0.46 of quercetin. These outcomes indicated that the hydroalcoholic leaf extract of Grewia hirsuta plant contains phytoconstituents that exhibit antioxidant activity possible because of the existence of bioactive compounds.

INTRODUCTION
The diseases are caused due to damage of tissues through active nitrogen, active oxygen species, as well as active chlorine, are byproducts of metabolism or entered the body from external sources (Cochrane, 1991). These “free radicals play a” main “role in the” procedure of aging as well as serious diseases like diabetes mellitus, cardiovascular diseases, cataract development, respiratory diseases, neurodegenerative disorders, rheumatoid arthritis, and various cancers. The three basic units of cells nucleic acids, lipids as well as proteins molecules damage owing to generation of...
Antioxidants are naturally occurring compounds that protect the body from oxidative free radicals and obtained from plants mainly are phenolic flavonoids, alkaloids, tannins, and phenolic compounds include phenolic acids, tocopherols, cinnamonic acids carotenoids as well as benzoic acid derivatives (Zargoosh et al., 2019). The plant origin chemical compounds have the potential to be replaced by synthetic drugs and reduce side effects than synthetic drugs (Berahou et al., 2007). Hence above 80% of developing or under rising populations primarily using herbal extracts or their active ingredients for health care purposes (Hasan et al., 2016).

Grewia hirsuta belongs to family Tiliaceae and usually known as nagbala (Sharma, 2013). Various parts of the plant traditionally used in the treatment of headache, vision problems, mouth sores and cholera (Goyal, 2012). A variety of phytoconstituents was isolated from plant Grewia hirsuta Vahl includes aldehyde, alcoholic compound, terpenes, undecanoic acid, myristic acid, and linolelaidic acid, “palmitic acid, linoleic acid, oleic acid” (Natarajan et al., 2015).

**MATERIALS AND METHODS**

**Plant Material**

Grewia hirsuta’s Fresh leaves have been collected from the forest near to Rani Channama University, Belgavi, (Latitude: 16.0073130, Longitude: 74.4968770) of Karnataka, India in November 2018. The fruits have been washed to take away foreign matters, and then shed dried. Plant identification and authentification was done as Grewia hirsuta Vahl by an expert taxonomist from “ICMR-National Institute of Traditional Medicine, Belagavi, Karnataka, India”.

**Preparation of plant extract**

The extraction" process followed is referred to as total a polar organic solvent extraction (Demirci and Gray, 2006). The dried and powdered leaves of 150 grams, thoroughly extracted with hydroalcoholic solvent (70:30: Methanol: Water) by maceration method and extract was evaporated above their boiling points Mukherjee (2019); Khandelwal (2008) (Mukherjee, 2019; Khandelwal, 2008; Gokhale, 2008).

**Qualitative Phytochemical Analysis**

The HAEGH was screened to recognize the existence of primary as well as secondary metabolites, like glycosides, terpenoids, alkaloids, flavonoids, tannins, saponins, proteins along with fixed oils, by phytochemical procedures and standard screening test (Roopashree et al., 2008; Adibe et al., 2009; Audu et al., 2007).

**Total phenol content estimation”(TPC)**

The TPC was calculated through the modified folin-ciocalteu technique. Gallic acid 10mg and Methanol 10ml used for the preparation of a standard sample. The test sample of HAEGH was prepared by adding 10mg of dried extract powder into 10ml methanol as
Table 1: Phytochemical analysis of Grewia hirsuta leaf extracts.

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>HAEGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for Alkaloids</td>
<td></td>
</tr>
<tr>
<td>Hager’s test ++</td>
<td></td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>Legal’s test ++</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Lead acetate</td>
<td></td>
</tr>
<tr>
<td>Phenols</td>
<td>++</td>
</tr>
<tr>
<td>Ferric Chloride Test</td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Xanthoproteic test</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
</tr>
<tr>
<td>Fehling’s test</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td></td>
</tr>
<tr>
<td>Froth Test</td>
<td>+</td>
</tr>
<tr>
<td>Diterpins</td>
<td></td>
</tr>
<tr>
<td>Copper acetate test</td>
<td>+</td>
</tr>
</tbody>
</table>

where, +: present (mild amount), ++: present (moderate amount), +++: present (large amount), –: absent, based on the power of generated “color reaction”.

Table 2: Estimation of total “phenol, “flavonoids” and alkaloid “content of” HAEGH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolic content (mg/100mg)</th>
<th>Total flavonoids content (mg/100mg)</th>
<th>Total alkaloid content (mg/100mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic extract of Grewia hirsuta leaf</td>
<td>3.627</td>
<td>4.059</td>
<td>5.671</td>
</tr>
</tbody>
</table>

Table 3: Percentage Inhibition of ascorbic acid and HAEGH using DPPH method.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Ascorbic acid</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>44.65</td>
<td>33.77</td>
</tr>
<tr>
<td>20</td>
<td>48.62</td>
<td>48.89</td>
</tr>
<tr>
<td>40</td>
<td>65.34</td>
<td>62.45</td>
</tr>
<tr>
<td>60</td>
<td>69.65</td>
<td>73.32</td>
</tr>
<tr>
<td>80</td>
<td>77.41</td>
<td>79.98</td>
</tr>
<tr>
<td>100</td>
<td>84.13</td>
<td>88.23</td>
</tr>
<tr>
<td>IC 50</td>
<td>17.681</td>
<td>25.903</td>
</tr>
</tbody>
</table>

well as filtrate. From filtrates 2ml solution of extract used for the estimation (Olajuyigbe and Afolayan, 2011).

Total flavonoids content estimation (TFC)

TFC was estimation based on aluminum chloride method. The standard solution is made by 10mg quercetin dissolved in 10ml methanol. The test solution prepared by 10mg of dried HAEGH was dissolved in 10ml methanol and filters it. From filtrate, 3ml was utilized for the evaluation of TFC (Moteriya et al., 2014).

“Total” alkaloids “content” estimation (TAC)

The principle of TAC is standing on response of alkaloid among bromocresol green solution that forms a yellow-colored solution. The dried 1mg plant extract mixed with “1ml of 2 N HCl” as well as “filtered. This solution” of pH adjusted by 0.1 N NaOH and poured into separating funnel. After that “5 ml of phosphate buffer and 5 ml of bromocresol green solution” had been included. The combi-
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Figure 4: Finger print of HAEGH and quercetin standard via win CATS Planar Chromatography Manager

Figure 5: Chromatogram for standard quercetin flavonol.

Figure 6: Chromatogram for flavonol from HAEGH.

Figure 7: Chromatograms obtained from separation of HAEGH. Visualization was under UV light of wavelength 254 nm.

Antioxidant activity of extract using DPPH’ method

The scavenging activity has been assessed through the spectrophotometer process. In this assay reference, standard and control were used butylated hydroxytoluene and methanol respectively. The principle involved evaluation of antioxidant capacity by the reduction in DPPH radicals that analyzed by transformation of colors of solution from deep violet to light yellow, measured at 517 nm wavelength. A stock solution for test prepared with 6 mg DPPH in 100 ml methanol and 1.5 ml of this solution combined by various concentrations of HAEGH. The absorbance in the presence of test extract at various “concentrations (10-100 µg/ml) was noted after 15 minutes. The control reading has been noted...

nution was shaken vigorously as well as extracted with chloroform. Then extracts have been transferred into a 10ml volumetric flask along with diluted through chloroform. Standard sample prepared by various aliquots “of atropine (40, 60, 80, 100 and 120 µg/ml)” have been organized similarly as described previously. The experiment, as well as standard sample absorbance, was calculated through a UV/Visible spectrophotometer at 470nm wavelength against the reagent blank (Fadhil et al., 2007).

HPTLC fingerprinting analysis

HAEGH extract of 100mg made soluble in 1 ml methanol along with centrifuged at 1000g for five minutes, this used as a test solution and quercetin utilized as standard for HPTLC analysis. The instrument used CAMAG Linomat 5 “Linomat5_171122” S/N 171122 (1.00.12) with Mobile stage- Toulene: Ethylacetate: Formic Acid: Methanol (5.5:3:1:0.5) and stationary material used “HPTLC plates silica gel 60 F 254 (E. MERCK KGaA). The Linomat 5 application parameters” include inert spray gas, methanol as solvent, 150nl/s dosage speed with 0.2 ul pre-dosage volume. The Sequence includes 100 µl syringe size, total number of tracks was 15 and application position Y: 8.0mm & Band length: 8.0mm. Detection of plates executed by CAMAG TLC Scanner “Scanner_171021” S/N 171021 (2.01.02) Position of initial track X 15.0mm, Distance among tracks 12.1 “mm, Scan start pos. Y 5.0mm, Scan end pos. Y 75.0mm, Slit dimensions 6.00 x 0.30mm, Micro, Optimize optical system Light, Scanning speed: 20 mm/s, Data resolution: 100 µm/step".

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through the adding up 1.5 ml of DPPH solution with makeup volume 3 ml with methanol and absorbance has been used directly at 517 nm. All tests were carried out thrice and mean taken for calculation. Recording of reduction in absorbance has been mentioned of DPPH through the sample at various concentration after 15 minutes at 517 nm (Olajuyigbe and Afolayan, 2011).

"Calculation of % Reduction" =
"Control Absorbance - Test Absorbance" × 100

RESULTS AND DISCUSSION

Estimation of Phytochemical Constituents*

The phytochemical investigation of HAEGH exhibited the existence of important biologically active secondary metabolites, like saponins, phenols, alkaloids, ditrepins, flavonoids, glycosides, carbohydrates, and proteins which were established that demonstrated in Table 1. That based on the intensity of the color reaction, the greatest amount of alkaloids, flavonoids, as well as glycosides, were contained by the HAEGH extract

Total phenol content estimation (TPC)

Gallic acid equivalent of TPC was expressed as mg/100mg of dry extract sample utilizing the equation acquired from the calibration curve: Y = 0.011X+0.011, R²= 0.998, in which Y stand for absorbance and X referred as gallic acid equivalent (GAE). The TPC was observed to be 3.627mg/100mg of HAEGH, which was shown in Table 2 and Figure 1.

Total flavonoids content estimation (TFC)*

TFC was evaluated as quercetin equivalent (mg/100mg) utilizing “the calibration curve: Y=0.040X + 0.009, R²=0.999, in which Y measured absorbance and X represent quercetin equivalent (QE). The TFC was found 4.059mg/100mg of HAEGH, which exposed in Table 2 and Figure 2.

Total alkaloid content estimation (TAC)

TAC was shown as atropine equivalent mg/100mg utilizing the equation stand on the calibration curve: Y=0.007X + 0.024, R²=0.995, in which Y measured as absorbance and X represented the Atropine equivalent (AE). The TAC was found 5.671mg/100mg of HAEGH, shown in Table 2 and Figure 3.

HPTLC fingerprint analysis

The HPTLC fingerprinting validates a total of fifteen peaks, peak one to seven represents HAEGH and peaks onwards seven revealed standard drug Quercetin. The sharp and compact peaks of Quercetin was obtained at Rf = 0.46 with absorbance mode at 254nm shown in Figures 4 and 5.

The results at wavelength 254nm for HAEGH revealed the presence of polyvalent phytoconstituents. The component with Rf value 0.44 was found to be superior with the percentage area is 89.41% exposed in Figure 6. The visualization of chromatogram obtained from HAEGH shown in Figure 7.

DPPH scavenging activity:

The study explored, HAEGH for their antioxidant potential with DPPH scavenging assay. Assay followed criteria IC₅₀ for interpretation of antioxidant activity. It was based on the linear regression equation which says the correlation between the concentration of the sample (X-axis) with the percent inhibition (Y-axis) shown 50% decreased activity of DPPH concentration. Percentage inhibition was estimated from the reduction of arithmetic DPPH absorbance with the absorbance of the sample.

Outcomes of the antioxidant activity of Grewia hirsuta hydroalcoholic extract compared to the ascorbic acid were revealed in Table 3. The hydroalcoholic extract established a high antioxidant activity compared against ascorbic acid.

Medicinal plants are beneficial sources of antioxidant compounds as well as reported “an important role in the treatment of diseases” worldwide (Fouche et al, 2015). Various plants have been shown antioxidant profiles like the reduction of DPPH radicals reducing power because of the existence of bioactive secondary metabolites that are rich in antioxidant along with “free radical scavenging” qualities (Kipkore et al, 2014). The plant Grewia hirsuta Vahl has conventionally been utilized through people in many region of India and various parts of a plant traditionally used in the treatment of headache, vision problems, sore throat, and cholera (Goyal, 2012). It was reported that the whole plant contains phytoconstituents like steroids, flavonoids, alkaloids, tannins (Yamazaki and Kawano, 2011).

The current study was based on conventional use along with preliminary phytochemical constituents to estimate the antioxidant profile of the hydroalcoholic extract of Grewia hirsuta Vahl. The plant leaves collected from the Western Ghats region of Karnataka province and the reasons for the collection of plants from a specific location that affects ecological factors. The ecological factors apart from species are climate, soil; along with location lead to changes in the development of medicinal plants moreover the quality and quantity of their active...
ingredients, like steroids, glycosides, alkaloids, as well as essential oils (Omidbeigi, 2012). A study published in *Nature Scientific Report* showed that an ecological factor-like elevation, climate, and soil features, etc produces considerable consequence on the potency of the extract, antioxidant actions as well as total phenol content of *Scrophularia striata* Boiss plant (Zargoosh et al., 2019).

One study reported the free radical scavenging activity of *Grewia hirsuta* whole plant, which was collected from the Nanmangalam forest, Chennai, India state that 200 μg methanol extract of plant exhibited 95 % free radical scavenging inhibition activity (Ema et al., 2013). But in the current study, it has been proved that the hydroalcoholic extract of plant leaves collected from the Western Ghats region of Karnataka province exhibited the better antioxidant activity at dose merely 100 μg with 88.23 % inhibition.

It was envisaged that the entire polyphenols content of hydroalcoholic extract of *Grewia hirsuta* leaf extract had been the foremost accountable parts for the antioxidant potentials, additionally, it has been confirmed that various ROS (reactive oxygen species) specifically, as superoxide anion, hydroxyl radicals and hydrogen peroxide” produced from the system had been inhibited through the different antioxidant phytoconstituents present in medicinal plant and preventing further cell damages (Yamunadevi et al., 2011). In DPPH assay, reduction of ferrie ion into ferrous ion through antioxidants phytoconstituents present in the hydroalcoholic extract, which develops a change in color, depending upon their concentration of phytoconstituents and reducing power capacity (Sravani and Paarakh, 2012).

HPTLC analysis reveals the presence of active constituents in the hydroalcoholic extract of *Grewia hirsuta* leaf was confirmed quercetin by peak but even though further study is required to confirm the identity of compounds.

**CONCLUSIONS**

The present study confirmed the hydroalcoholic extract obtained from the leaf of *Grewia hirsuta* (family: *Tiliaceae*) plant collected from the Western Ghats forest, possesses significant antioxidant activity. The HPTLC fingerprint analysis showed the presence of quercetin compound as compared to the standard quercetin chromatograph. The plant extracts could be invoked as a renewable source of natural safe antioxidants in nutraceutical preparations or food supplements. Further study is under process to confirm the identity of the actual component present in hydroalcoholic extract responsible for the antioxidant property.

**ACKNOWLEDGEMENT**

The authors are grateful to Dr. Prabhat Jain, Scan Research Laboratory, Bhopal, MP for their support to conduct analytical work.

**Conflict of Interest**

The authors declare no conflicts of interest.

**Funding Support**

None.

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