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# Phytochemical Analysis and Determination of Antioxidant Activity of Methanolic Leaf Extract of *Gossypium herbaceum* (Levant Cotton)

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## Abstract

Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Plant *Gossypium herbaceum* plays a crucial role in folk medicine. The present study was conducted with the main objectives of qualitative, quantitative phytochemical analysis and evaluation of antioxidant properties methanolic leaf extract of *G. herbaceum*. Leaves of *G. herbaceum* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with methanol. Results depicted that the major phytochemicals found in methanolic extract of leaves of *G. herbaceum* were found to be alkaloids, flavonoids, proteins & amino acids, reducing

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sugars, saponins, phenolic compounds/tannins, and terpenoids. Quantitative estimation of phytochemicals in methanolic extract of leaves of *G. herbaceum* revealed that total phenolic quantity was found to be highest (51.36 GAE) followed by total flavonoid (45.28 GAE), and Tannins (3.68 mg/mL). The $IC_{50}$ values exhibited by methanolic extracts of leaves of *G. herbaceum* and standard ascorbic acid was found to be 39.44 µg/mL and 11.98 µg/mL respectively. In conclusion, biological activity such as antioxidant properties of methanolic extracts of leaves of *G. herbaceum* depicted that *G. herbaceum* could be potential drug agent of folk medicines.

**Keywords:** *Gossypium herbaceum*, Leaves, Methanolic extract, Antioxidant, Total phenols

## Introduction

Chemicals derived from plants have recently attracted a lot of attention due to their wide variety of medicinal applications. Medicinal plants are collection of species that accrue variety of active ingredients that are effective in treating a range of human and animal ailments. They are the most abundant bio-resource for medications used in traditional and modern medical systems, as well as for nutraceuticals, food supplements, traditional remedies, pharmaceutical intermediates, and chemical building blocks for synthetic drugs.[1] Phytochemicals are naturally occurring in different parts of the medicinal plants that have defense mechanism and offer protection from a variety of ailments.[2] The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents which produce definite physiological action on the human body and these bioactive substances include alkaloids, carbohydrates, terpenoids, steroids, flavonoids, tannins, etc.[3]

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Free radicals are continuously being produced in our body as a result of various metabolisms. Some amount of free radicals are very much necessary for body host defense system, signalling mechanism and in the induction of a mitogenic response. But the persistence of these free radicals even after their activity results in deleterious effects. These free radicals act on important biomolecules like nucleic acids (mutations), lipids (membrane lipid peroxidation), proteins (oxidation) and carbohydrates resulting in various diseases. Over 70 degenerative diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), memory loss, depression, arthritis, cancer, ageing etc..) are caused due to free radicals.[4-6] Herbal medicines are the materials which are derived from one or more plant which possess some curative values to prevent human body from common diseases.[7] Free radical scavenging property can be removed completely by the antioxidants and maintains the balance in the body.[8]

Malvaceae is a family of flowering plants containing 243 genera and at least 4,225 species of herbs, shrubs, and trees. Economically, the most important member of the family is *Gossypium* (cotton).[9] *Gossypium herbaceum* plant (Figure 1) is mentioned in indigenous systems of medicine. It is an erect, shrubby, hairy plant, 2-8 feet high with thick woody stem and twigs and leaves sparsely hairy, rarely glabrous. The leaves are 5-7 lobed, lobes ovate, and rotundus only slightly constricted at base.[10,11] Bracteoles with 6-8 serrated teeth on the margin, broadly triangular, usually broader than long. The flowers are large, yellow with purple center; calyx base is black with glandular dots and capsules ovate, pointed. The part of the plant used in medicine are seeds, [10,11] leaves,[11] root,[10,11] and root bark.[11] The leaves are emollient, mucilaginous, haematinic, diuretic, cooling, constipating and used in gastric irritation, diarrhoea, dysentery, dysuria, rheumatoid arthritis and Otalgia.[12]

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Figure 1: Showing *Gossypium herbaceum* plant

*G. herbaceum* is reported as traditional medicine plant with the unique properties like neurotonic for memory and learning,[13] Antiepileptic,[14] Antioxidant,[15] Antidiabetic,[16] Antihyperlipidemic,[17] wound healing,[18] Antimicrobial,[19] Diuretic,[20] Ulcer healing.[21] With this background, the present study was designed with the objectives of phytochemical analysis and determination of antioxidant activity of leaf part of *G. herbaceum*.

## Materials and Methods

### Collection of plant material

The leaves of *G. herbaceum* were collected from the agricultural fields in Chikkaballapura, Karnataka, India. The leaves were prayed with ethanol, and then shade dried at room temperature for 10 days. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.[22,23]

### Extraction

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Approximately 50 g of dried and coarsely powdered leaves of *G. herbaceum* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of methanol. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.

**Phytochemical Screening**

Chemical screening was carried out on the methanolic extract of leaves of *G. herbaceum* by using standard procedure to detect constituents as described by sofora,[24] Trease and Evans[25] and Harborne.[26]

**Test for Alkaloids**

Approximately 0.2g of methanolic extract of leaves of *G. herbaceum* was warmed with 2% $H_2SO_4$ (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

**Test for Tannins and Phenolic Compounds**

The methanolic extract of leaves of *G. herbaceum* in **s**mall quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins.

**Test for Glycosides**

About 0.6g of methanolic extract of leaves of *G. herbaceum* was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

**Test for Reducing Sugars**

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The methanolic extract of leaves of *G. herbaceum* was shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

**Test for Saponins**

About 0.2g of methanolic extract of leaves of *G. herbaceum* was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

**Test for Flavonoids**

0.2g of methanolic extract of leaves of *G. herbaceum* was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

**Test for Steroids**

2 mL of acetic anhydride was added to 0.5g methanolic extract of leaves of *G. herbaceum* and then added 2 mL of $H_2SO_4$. The change of color from violet to blue or green or red showed the presence of steroids.

**Test for Terpenoids**

0.3g methanolic extract of leaves of *G. herbaceum* was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M $H_2SO_4$ was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

**Test for Proteins and Amino acids**

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To the 0.3g methanolic extract of leaves of *G. herbaceum* few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue colouration indicate the presence of proteins.

**Quantitative Estimation of Phytochemicals**

**Total phenolics**

The concentration of total phenolics in the methanolic extract of leaves of *G. herbaceum* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[27] The phenolic content of the extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

**Total flavonoid**

Aluminum chloride colorimetric method was used for flavonoids determination for methanolic extract of leaves of *G. herbaceum*.[28] The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

**Total Tannins**

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of methanolic extract of leaves of *G. herbaceum* was added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5 mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

**Antioxidant Assay**

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The modified literature protocol of Blois was used for antioxidant assay.[29,30] Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) in methanol. The control was also run which contains only methanol. The hydrogen atom or electron donation abilities of extract and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control) × 100. The $IC_{50}$ value was determined by using linear regression equation *i.e.*, Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trendline.

**Results**

The major phytochemicals found in methanolic extract of leaves of *G. herbaceum* were, found to be alkaloids, flavonoids, proteins & amino acids, reducing sugars, saponins, phenolic compounds/tannins, and terpenoids (Table 1).

**Table 1:** Photochemical screening of methanolic leaf extract of *G. herbaceum*

| Chemical Components | Methanolic Extract of leaves of G. herbaceum |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Glycosides | - |
| Proteins and Amino acids | + |
| Reducing sugar | + |

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| | |
|---|---|
| Saponins | + |
| Steroids | - |
| Phenolic compounds | + |
| Tannins | + |
| Terpenoids | + |

Moreover, quantitative estimation of phytochemicals in methanolic extract of leaves of *G. herbaceum* revealed that total phenolic quantity was found to be highest (51.36 GAE) followed by total flavonoid (45.28 GAE), and Tannins (3.68 mg/mL) (Table 2).

**Table 2:** Quantitative estimation of phytochemicals present in methanolic leaf extract of *G. herbaceum*

| **Chemical Components** | **Methanolic Extract of Leaves of *G. herbaceum*** |
|---|---|
| Total phenolics | 51.36 GAE |
| Total flavonoids | 45.28 GAE |
| Tannins | 3.68 mg/mL |

Furthermore, the $IC_{50}$ values exhibited by methanolic extracts of leaves of *G. herbaceum* and standard ascorbic acid was found to be 39.44 µg/mL and 11.98 µg/mL respectively (Table 3).

**Table 3:** Antioxidant activities of methanolic leaf extracts of *G. herbaceum*

| **S. No.** | **Sample** | **$IC_{50}$ (µg/mL)** |
|---|---|---|
| 1 | Leaf Extract | 39.44 |

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| | | |
|---|---|---|
| 2 | Ascorbic Acid | 11.98 |

**Discussion**

Concerns over the safety of synthetic antioxidants have shifted the global interests towards exploration of antioxidant compounds from natural sources. A plethora of phenolic compounds extracted from several plant species have been reported to possess strong antioxidant activities. Phenolic compounds are ubiquitously present in plants, and when plants are consumed as foods, these phytochemicals contribute to the intake of natural antioxidants in the diets of human as well as animals. Out of all the phenolics, the flavonoids belong to a large family of compounds with a common diphenyl propane structure with different degree of hydroxylation, oxidation and substitution. These compounds commonly occur as glycosides in plants and are reported to be most diverse and efficient as antioxidants.[31] Hence, in the current study we aimed for qualitative and quantitative phytochemical analysis and determination of antioxidant activity of methanolic leaf extracts of *G. herbaceum*.

The qualitative phytochemical analysis of methanolic leaf extracts of *G. herbaceum* revealed the presence of alkaloids, flavonoids, proteins & amino acids, reducing sugars, saponins, phenolic compounds/tannins, and terpenoids phytochemicals. The qualitative phytochemical analysis of methanolic leaf extracts of *G. herbaceum* revealed that total phenolic quantity was found to the major phytochemicals found in methanolic leaf extracts of *G. herbaceum.* The estimation of antioxidant activities of methanolic leaf extracts of *G. herbaceum* revealed $IC_{50}$ values exhibited by methanolic extracts of leaves of *G. herbaceum* and standard ascorbic acid was

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found to be 39.44 µg/mL and 11.98 µg/mL respectively based on in-vitro DPPH radical scavenging method.

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular disease, inflammatory conditions, cancer and ageing.[32] Antioxidant may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent disease.[33] The 1, 1 - diphenyl –2– picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or crude mixtures such as the ethanol extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity was increased with increasing percentage of the free radical inhibition. DPPH is relatively stable radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution loses color stochiometrically depending on the number of electrons taken up.[29]

Kumar et al., reported that the hydro-alcoholic leaf extract of *G. herbaceum* showed decreases the free radical to correlative with hydrazine when it reacts with hydrogen donors in anti-oxidant principle. Furthermore, authors corroborated the anti-oxidant activity with respect to its phenolic content.[34] Concurrently, methanolic extract of *G. herbaceum* showed high phenolic content in our study and hence DPPH free radical scavenging activities of methanolic extract of *G. herbaceum* could be accredited to total phenolic content.

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Summarily, results obtained in the present study are encouraging as this study evidenced the vide variety of secondary metabolites present in the methanolic leaf extracts of *G. herbaceum* and methanol fractions of *G. herbaceum* have shown considerable antioxidant properties. Hence this study supplies as evidence-based study for leaf of *G. herbaceum* could be exploited in the management of various ailments.

## Conclusions

This study confirms that the *G. herbaceum* has vide variety of secondary metabolites. Biological activity such as antioxidant properties of methanolic leaf extracts of *G. herbaceum* depicted that *G. herbaceum* could be potential drug agent of folk medicines. However further studies need to be conducted to elucidate the mechanism of action of various secondary metabolites present in *G. herbaceum* against various ailments.

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