Phytochemical Investigation and Antioxidant Activity of Successive Solvent Extracts of Celosia argentea Leaves

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ABSTRACT

Oxidative stress has been one of the key factors to provoke chronic and degenerative disorders, and this has created more interest in finding safe and effective natural antioxidants plants. The aim of the current work was to determine the phytochemical properties and antioxidant activity of successive solvent extracts of Celosia argentea leaves. Successive solvent extraction using solvents of increasing polarity was used on dried leaf powder. Extracts obtained were analyzed in terms of extraction yield, qualitative phytochemical composition, total phenolic content (TPC), total flavonoid content (TFC), and in-vitro antioxidant activity in terms of DPPH, ABTS and FRAP test.

The outcome indicated that the extraction pattern is polar, and the best yield was realized in the ethanolic extract, then ethyl acetate extract, and chloroform extract. Qualitative phytochemical screening revealed the existence of several bioactive compounds such as phenolics, flavonoids, alkaloids, tannins, and glycosides that were mainly found in medium to highly polar extracts. The quantitative analysis indicated that the ethanolic extract had the highest values of TPC and TFC, and non-polar extracts had the lowest values. Antioxidant analysis indicated that polar extracts had high free radical clearing and reducing capacity and the ethanol extract performed the best in all antioxidants during the analysis.

Keywords: Celosia argentea, antioxidant activity, phytochemical screening, total phenolic content, total flavonoid content.

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1. INTRODUCTION

Oxidative stress is a basic biological process which occurs as a result of the excess or deficiency between the generation of reactive oxygen species (ROS) and the ability of endogenous antioxidant defense systems to counteract it. Reactive oxygen species, superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen are continually produced as a normal cellular metabolic process i.e., in mitochondrial respiration, inflammatory process, and enzyme reactions. These reactive species are significant to the cellular signaling and homeostasis in physiological conditions. Nevertheless, a high level of ROS production or loss of antioxidant defenses may cause oxidative stress, which causes damage to cellular macromolecules, including lipids, proteins, and nucleic acids [1-3].

Oxidative stress has been shown to have a significant role in the pathogenesis and pathophysiology of many chronic and degenerative diseases. ROS-induced lipid peroxidation decreases the membrane integrity and fluidity, which results in disrupted cellular permeability and dysfunction. Receptor dysfunction, enzyme inactivation, oxidative alteration of receptors, and signal transduction pathways, as well as mutations, genomic instability and apoptosis caused by oxidative DNA damage [4,5]. Therefore, oxidative stress has been implicated as a cause of diverse diseases such as cancer, cardiovascular diseases, diabetes mellitus, neurodegenerative disorders like Alzheimer and Parkinson disease, inflammatory diseases and premature aging [6-8]. The increasing prevalence of oxidative stress-related diseases has increased the need to seek practical antioxidant solutions that can help in restoring redox homeostasis to prevent cell damage.

Antioxidants are the materials that are capable of delaying, preventing, or inhibiting oxidative injury by scavenging free radicals, metals, or increasing endogenous antioxidant protective mechanisms. Synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole have been extensively used in the pharmaceutical industry and the food sector but issues of their long time safety, toxicity and carcinogenicity has curtailed their usage [9,10]. This has led to an overwhelming change in the search to discover natural antioxidants with plant origin, which is considered to be much safer and more biocompatible in the long run.

Medicinal plants are an excellent source of natural antioxidant-containing compounds, such as phenolics, flavonoids, alkaloids, tannins, terpenoids and glycosides. Those phytochemicals possess antioxidant effects that are mediated in a variety of mechanisms which include direct free radical scavenging, inhibition of lipid peroxidation, regulation of antioxidant enzymes, and inhibition of pro-oxidant pathways [11-13]. Plant-derived antioxidants may work synergistically unlike single-molecule synthetic antioxidants, generating increased biological activity and expanded protective effects. Both epidemiological and experimental research has persistently shown that plant-based antioxidant-rich diets are related to a lower chance of developing chronic illnesses that are connected to oxidative stress [14,15].

Of the antioxidants found in plants, the phenolic compounds and flavonoids have been of specific interest because of their high redox potential, and their capacity to either donate hydrogen atoms or electrons, and neutralize free radicals. The total phenolic and total flavonoid content of plant extracts is often implicated in the antioxidant activity of plant extracts and as such, they are significant parameters to determine its biological activity [16,17]. This has led to quantitative determination of the phenolics and flavonoids, and in-vitro antioxidant activity, including DPPH, ABTS, and FRAP, as a common method of analysing the antioxidant potential of medical plants.

The extraction is a key factor in yield, composition and biological activity of antioxidants of plant origin. Polar solvents have a powerful effect on the phytochemical solubility and recovery. Serial solvent extraction with increasingly polar solvents is a conventional method of fractionating plant material into chemically separate extracts that are concentrated enriched with certain groups of bioactive compounds [18,19]. Lipids and waxes are usually extracted by non-polar solvents with the more polar solvents favoring phenolics, flavonoids, and glycosidic compounds. Sequential solvent extracts can thus be comparatively evaluated to give useful information on the distribution of antioxidant constituents and be used to identify bioactive fractions that are more active.

Amaranthaceae, Celosia argentea is a significant medicinal plant that has been widely used within the traditional medicine systems of diverse disorders in the context of the treatment of several illnesses. The various components of the plant especially leaves have been documented to have antimicrobial, anti-inflammatory, antioxidant, hepatoprotective and wound-healing effects [20-22]. The conventional use and early pharmacological research indicates that Celosia argentea leaves are endowed with bioactive phytoconstituents thus making it an attractive antioxidant candidate.

The phytochemical studies of Celosia argentea have shown that it contains phenolic compounds, flavonoids, alkaloids, saponins, tannins, glycosides, and many of them are reported to have antioxidant properties [23-25]. The majority of published research, however, does not investigate solvent extracts in their crude form, but only on the antioxidant potential of solvent extracts as a whole. The crude extracts are complex mixtures of the active and inactive constituent and may thereby conceal or dilute the activity of a strong bioactive compound.

Phytochemical complexity is better resolved through systematically analyzing successive solvent extracts; which makes it possible to identify fractions that are enriched with antioxidant constituents. The extraction yield, phytochemical composition, and antioxidant activity in the solvent fractions can be compared and clear correlations between the solvent polarity, phytochemical abundance, and biological activity may be established [26-28]. These kinds of studies based on fractions are crucial in giving reasonable choices of lead extracts to be used in further pharmacological and mechanistic studies.

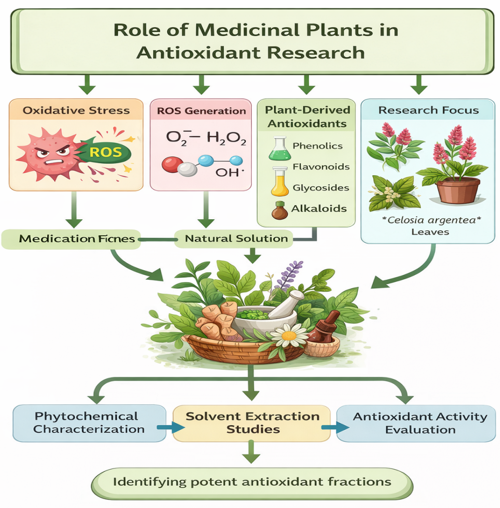

Figure 1: Role of Medicinal Plants in Antioxidant Research with Reference to *Celosia argentea* Leaves

2. MATERIALS AND METHODS

2.1 Materials

Celosia argentea was used to gather fresh leaves of the plant at the right time. Analytical grade petroleum ether, chloroform, ethyl acetate and ethanol were the solvents of extraction employed. Phytochemical screening and antioxidant assays reagents, including Folin-Ciocalteu reagent, aluminum chloride, DPPH, ABTS, potassium persulfate, ferric chloride, TPTZ, sodium carbonate, and common commercial reagents, including gallic acid and quercetin, were purchased by a local laboratory supplier. The chemicals were taken without any purification.

2.2 Methodology

2.2.1 Plant authentication

A Qualified taxonomist was used to identify the plant material collected depending on its macroscopic and taxonomic features. A specimen of the voucher was put up in the institutional herbarium to be used in the future. The leaves were dried to remove moisture content, dried at room temperature and shade-dried to avoid decomposition of phytoconstituents and finally, coarse powdering was performed on a mechanical grinder to ensure the authenticity of leaves. The powder was put in airtight containers until further use [29,30].

2.2.2 Successive solvent extraction

Subsequent solvent extraction was done in information of increasing information of solvents to provide exhaustive circumference of phytochemicals. Soxhlet extraction of the powdered leaf material was done consecutively using petroleum ether, chloroform, ethyl acetate, and ethanol. Each extraction was continued until siphon solvent turned colorless. Rotary evaporator was used to filter the extracts and concentrate them in reduced pressure. The hide extracts were dried, then weighed and kept in desiccators until analysis [31-33].

2.2.3 Phytochemical screening

Qualitative phytochemical screening of all extracts Preliminary screening of all extracts with standard chemical tests was conducted to identify major classes of secondary metabolites like alkaloids, flavonoids, phenolics, tannins, saponins, glycosides and terpenoids. The emergence of the typical color changes or the emergence of the precipitate was considered as a sign of the presence of certain phytochemical groups [29,34].

2.2.4 Total phenolic and total flavonoid content estimation

Folin-Ciocalteu colorimetric method was used to determine the total phenolic content (TPC). The reaction mixture was then measured and the absorbance at the wavelength of 765 nm recorded, and the results were reported in terms of milligrams of the gallic acid equivalent per gram of extract. The amount in total flavonoid content (TFC) was determined by the colorimetric method based on the presence of aluminum chloride and the absorbance at 415 nm. The amount of flavonoids was estimated in terms of milligrams of quercetin equivalents per gram of extract [35,36].

2.2.5 Antioxidant Activity

2.2.5.1 DPPH radical scavenging assay

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the free radical scavenging activity of the successive solvent extracts. Freshly prepared DPPH methanolic solution had a deep violet hue because of free radicals that were present in the solution. The specified period was used to allow the reaction to take place by mixing different concentrations of each extract with the DPPH solution and incubation in the dark at room temperature. Antioxidant activity of the extracts was determined by the decrease in DPPH radicals which in turn reduced absorbance that was spectrophotometrically measured at 517nms with an apposite blank. The radical scavenging activity percentage was calculated and the antioxidant potential of the extracts was in terms of the IC50 values of extracts which are expressed as the concentration that inhibits 50 percent of DPPH radicals [37-39].

2.2.5.2 ABTS radical cation scavenging assay

The ABTS radical cation scavenging of the extracts was evaluated by the ABTS+ assay. To obtain the ABTS radical cation, potassium persulfate was added to the ABTS solution after which the mixture was allowed to rest in the dark to ensure that a stable blue-green colored radical solution was obtained. The radical solution was thinned and a solvent to a point of attaining an absorbance of about 0.70 at 734 nm. Each extract was aliquoted and then added to the ABTS+ solution at various concentrations and then the reduction in absorbance was spectrophotometrically measured at 734 nm after incubating it. The scavenging ability of the extracts was determined by the percentage of ABTS radicals inhibited [37-39].

2.2.5.3 Ferric reducing antioxidant power (FRAP) assay

To determine the reducing capacity of the extracts in terms of their capability to reduce the ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, the ferric reducing antioxidant power (FRAP) assay was carried out. FRAP reagent was prepared freshly through the combination of acetate buffer, ferric chloride solution and 2, 4, 6- tripyridyl-s- triazine (TPTZ) solution. The FRAP reagent was measured and then added to the extract to incubate at regulated temperature. A blue colour of ferrous-TPTZ complex was followed by measuring the absorbance at 593 nm in a UV-Visible spectrophotometer. The more the absorbance value, the higher the reducing power of the extracts meaning the antioxidant potential [37-39].

2.2.6 Statistical analysis

Every experiment was conducted three times and the findings were given in the form of mean +- standard deviation (SD). Statistical evaluation was done by the method of one-way analysis of variance (ANOVA) after which suitable post-hoc tests were done. The level of 0.05 was taken as significant [40].

3. RESULTS AND DISCUSSION

The yield profile of sequential solvent leaf extracts of Celosia argentea leaves showed a distinct relationship on the polarity of the solvent (Table 3.1). Ethanolic extract recorded the best yield and subsequent results were recorded with ethyl acetate and chloroform extracts with the lowest yield registered with petroleum ether extract. This trend means that Celosia argentea leaves have high ratios of polar and medium polar phytoconstituents and non-polar elements in lesser amounts. This polarity-sensitive extraction pattern is typical of phenolics, flavonoids and glycosidic compounds which are soluble only in polar solutions.

Qualitative phytochemical screening showed that there were significant differences in the phytochemical compositions of the various extracts (Table 3.2). The petroleum ether extract had the least phyto chemical occurrence as compared to ethyl acetate and ethanolic extracts which had a wide range of secondary metabolites such as phenols, flavonoids, tannins, glycosides and alkaloids. The increasing polarity of the solvents in this progressive increase of phytochemicals is in support of the yield pattern of Table 3.1 and offers a biochemical rationale to further evaluation of the antioxidants.

These results were further supported by quantitative estimated values of total phenolic content (TPC) and total flavonoid content (TFC) (Table 3.3). The ethanolic extract recorded the highest levels of TPC as well as TFC, ethyl acetate extract rank second and petroleum ether extract registered the lowest. The solubility of phenolics and flavonoids is largely related to the solvent polarity and substantiates that polar solvents better accumulate antioxidant phytochemical relevant phenolics and flavonoids.

The ranking of antioxidant performances was a good indication of the phytochemical distributions in the extracts (Table 3.4). The ethanolic extract was the best antioxidant in terms of DPPH, ABTS, and FRAP and was also ranked first and second respectively, with ethyl acetate extract performing strongly. Chloroform extract had moderate antioxidant activity whereas petroleum ether extract had weak activity. The similarity in the high phenolic and flavonoid content (Table 3.3) and the high antioxidant activity in Celosia argentea leaf extracts (Table 3.4) implies the contribution of phenolic and flavonoid compounds as the main antioxidant activity of the extracts.

3.1 Yield Profile

Table 3.1: Percentage Yield of Successive Solvent Extracts of *Celosia argentea* Leaves

Sr. No.	Solvent Used	Nature of Solvent	Yield (% w/w)
1	Petroleum ether	Non-polar	Low
2	Chloroform	Low polarity	Moderate
3	Ethyl acetate	Medium polarity	Higher
4	Ethanol	Polar	Highest

3.2 Phytochemical Composition

Table 3.2: Qualitative Phytochemical Screening of Successive Solvent Extracts

Phytochemical	Petroleum ether	Chloroform	Ethyl acetate	Ethanol
Alkaloids	–	+	+	+
Flavonoids	–	–	+	+
Phenolics	–	+	+	+
Tannins	–	–	+	+
Saponins	–	–	–	+
Glycosides	–	–	+	+
Terpenoids	+	+	+	+

(+: Present, –: Absent)

3.3 Phenolic and Flavonoid Distribution

Table 3.3: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Extracts

Extract	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Petroleum ether	Low	Low
Chloroform	Moderate	Moderate
Ethyl acetate	High	High

Ethanol	Highest	Highest

3.4 Antioxidant Performance Ranking

Table 3.4: Comparative Antioxidant Activity of Successive Solvent Extracts

Extract	DPPH Activity	ABTS Activity	FRAP Reducing Power	Overall Rank
Petroleum ether	Weak	Weak	Low	IV
Chloroform	Moderate	Moderate	Moderate	III
Ethyl acetate	Strong	Strong	High	II
Ethanol	Strongest	Strongest	Highest	I

4. CONCLUSION

The current research proved that successive solvent extraction was efficient in the determination of phytochemical productivity and antioxidant capacity of Celosia argentea leaf extracts. Polar and medium-polar extracts, especially the ethanolic extract, had better extraction yield, rich phytochemical composition, higher levels of phenolic and flavonoid and better antioxidant activity. The similarity in the relationship between the abundance of phytochemicals and antioxidant activity is a good indication that Celosia argentea leaves hold potential as a natural source of antioxidants. These results confirm the choice of polar extracts in further bioactivity-directed and therapeutic studies.

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