RESEARCH ARTICLE

Phytochemical Profile and Antioxidant Activity of Medicinal Plant Extracts: Insights into Potential Therapeutic Applications

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ABSTRACT

This study evaluates the phytochemical composition and *in-vitro* antioxidant activities of various extracts such as *Boswellia serrate (Bs), Zingiber officinale (Zs), Tribulus terrestris (Tt), Camellia sinensis (Cs), Withania somnifera (Ws)*, and *Piper longum (Pl)*. Using soxhlet extraction, the plants were processed with various solvents, including n-hexane, dichloromethane, methanol, ethanol, and water. Phytochemical screening revealed a diverse range of compounds, such as alkaloids, flavonoids, and saponins, varying by extract. The antioxidant activities were assessed using superoxide, hydroxyl radical scavenging assays, and lipid peroxidation assays. The hydroalcoholic extract of *C. sinensis* exhibited the highest antioxidant activity, with significant superoxide and hydroxyl radical scavenging capabilities. *T. terrestris* and *W. somnifera* also demonstrated strong antioxidant properties, aligning with their traditional medicinal uses. Moderate activity was observed in *P. longum*, attributed to its bioactive compound, piperine. Meanwhile, *B. serrata* and *Z. officinale* showed lower antioxidant activities but are noted for their anti-inflammatory benefits. This research corroborates previous findings on the antioxidant potential of these plants, highlighting the importance of further investigations into their pharmacological applications. The study underscores the therapeutic potential of these extracts in oxidative stress-related conditions and supports their continued exploration in modern medicine.

Keywords: Antioxidant activity, Phytochemical screening, Medicinal plants, Herbal extracts, Free radical scavenging.

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INTRODUCTION

It is estimated that 80% of the world's population still relies on herbal remedies to treat diseases. Plant-derived materials are being investigated by major pharmaceutical companies for their potential therapeutic uses as a result of the growing global interest in herbal products. This trend led the WHO to emphasize in 2000 the importance of verifying herbal product's effectiveness and safety. The use of herbal medicines is becoming increasingly popular because they are considered safe alternatives and are often associated with fewer side effects.[1-3] As herbal medicines have become commercialized, concerns over their quality, safety, and efficacy have grown. A variety of factors, including plant identity and seasonal changes, can affect herbal raw materials. In order to improve the quality of herbal products and to reduce risks, the World Health Organization (WHO) has developed guidelines.[4-6] In this study, various extracts were evaluated *in-vitro* for phytochemical screening and antioxidant activities.

MATERIALS AND METHODS

Plant and Extraction Process

Various crude drugs of *Boswellia serrate (Bs), Zingiber officinale (Zs), Tribulus terrestris (Tt), Camellia sinensis (Cs), Withania somnifera (Ws)*, and *Piper longum (Pl)* were collected from a local area and a botanist confirmed it. With the use of a soxhlet apparatus, approximately 1-kg of each powdered dried plant material was extracted using solvents such as N-hexane, dichloromethane, methanol, and ethanol. Under reduced pressure, all traces of the solvents were removed from the extract. A vacuum desiccator was used to store the extracts.

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Determination of Phytoconstituents

The dried extracts were subjected to a comprehensive analysis to identify various chemical constituents as per standard procedure.[7]

***In-vitro* Antioxidant Activity**

The following three assay methods were used to determine *in-vitro* antioxidant activity in accordance with previously reported procedures.[8]

Superoxide Radical Scavenging Activity

A reaction mixture was assembled with the following components of 0.5 mL of 100 mm phosphate buffer (pH-7.4), 1-mL of 0.4 mM NADH, 1-mL of 0.156 mM NBT, 0.1 mL of 0.06 mM PMS, and 3 mL of an extract or standard drug (ascorbic acid) at concentrations ranging from 10 to 50 µg/mL, dissolved in 90% ethanol. The mixture was incubated at 25°C for 1 hour. To quantify the amount of formazan formed, the absorbance at 560 nm was measured after incubation.[9]

Hydroxyl Radical Scavenging Activity

To prepare the reaction mixture, 0.1 mL of 10 mM 2-deoxy -2-ribose, 0.33 mL of 50 mM phosphate buffer (pH-7.4), 0.1 mL of 0.1 mM $FeCl_3$, 0.1 mL of 0.1 mM ethylene diamine tetra acetic acid, 0.1 mL of 1 mM H_2O_2, 0.1 mL of 1-mM ascorbic acid and 1-mL of test sample at concentration ranging from 5 to 50 µg/mL were mixed. For 45 minutes, the mixture was incubated at 37°C. A solution of 2.8% (v/v) TCA acid and 0.5% (v/v) TBA, 0.5% v/v in 0.025% mol/L NaOH with 0.2% w/v butylated hydroxy anisole was then added. A pink chromogen was produced by heating this mixture for 15 minutes at 95°C. A blank solution was used to determine the absorbance at 532 nm after cooling.[10]

Lipid peroxidation scavenging assay

A 0.5 mL reaction mixture was prepared by combining 0.1 mL of 25% w/v rat liver homogenate in 40 mM tris-HCL buffer (pH-7.0), 30 mM KCl, 0.16 mM $FeCl_3$, and 0.06 mM ascorbic acid. This mixture was incubated at 37°C for 1-hour with and without the addition of the test sample/standard drug at varying concentrations (50–250 µg/mL). In previous study described a method for measuring thiobarbituric acid reactive substances to assess lipid peroxide formation. After incubation, 0.4 mL of the mixture was mixed with 0.2 mL sodium dodecyl sulfate, 1.5 mL thiobarbituric acid, and 1.5 mL 20% acetic acid (pH 3.5). Distilled water was added to the mixture and it was heated in a water bath at 100°C for 1-hour. A mixture of n-butanol and pyridine (10:1 v/v) was added after cooling to 1-mL of distilled water. After shaking vigorously and centrifuging for 10 minutes at 4000 rpm, the mixture was concentrated. As a result, the absorbance at 532 nm of the butanol-pyridine layer was measured to determine the amount of TBARS present.

The percentage inhibition of scavenging activity was calculated by following the equation.

$$\% \, inhibition = \frac{Acontrol - A \, test}{A \, control} x100$$

Where,

$A_{control}$= Absorbance of Control

A_{test}= Absorbance of test compounds (Sample/Standard)

Statistics

The Data were calculated as Mean ± SEM, based on triplicate values

RESULTS

Phytochemical screening of six different plant extracts revealed varying chemical constituents, as shown in Table 1. The n-hexane extract of *Boswellia serrata* contained steroid and triterpene compounds but was devoid of carbohydrates, alkaloids, glycosides, tannins, volatile oils, fats and fixed oils, flavonoids, polyphenols, saponins, amino acids, and gums and mucilage. There was no evidence of carbohydrates, glycosides, tannins, volatile oils, fats, fibers, polyphenols, saponins, amino acids, or gums and mucilage in the dichloromethane extract of *Zingiber officinale*. There were alkaloids, glycosides, steroids, triterpenoids, flavonoids, and saponins in the methanol extract of *Tribulus terrestris*, but no carbohydrates, tannins, volatile oils, fats and fixed oils, polyphenols, amino acids, or gums and mucilage. *Camilla sinensis* hydroalcoholic extract contains carbohydrates, alkaloids, glycosides, tannins, flavonoids, polyphenols, saponins, and amino acids, but not steroids, triterpenoids, volatile oils, fats, or gums. Carbohydrates, alkaloids, steroids, triterpenoids, flavonoids, and saponins were found in *Withania somnifera's* aqueous extract, but glycosides, tannins, volatile oils, fats and fixed oils, polyphenols, amino acids, and gums and mucilage were absent. Lastly, all other compounds tested in the aqueous extract of *Piper longum* were negative except for carbohydrates and alkaloids. As a result of this screening, it is evident that these medicinal plant extracts have a variety of chemical profiles.

***Invitro* Antioxidant Activity**

Superoxide radical scavenging activity

The results shown in Table 2 and Figure 1 indicate that all extracts demonstrated a concentration-dependent increase in scavenging activity. The hydroalcoholic extract of *C. sinensis* (HAECS) showed the highest activity, with a maximum inhibition of 73.79 ± 0.591% at 50 µg/mL. This was followed by the aqueous extract of *P. longum* (AEPL) at 71.53 ± 0.135% and the methanol extract of *T. terrestris* (METT) at 69.90 ± 0.091%. The n-hexane extract of *B. serrata* (NHEBS) and the aqueous extract of *W. somnifera* (AEWS) displayed similar activities, reaching 69.63 ± 0.125 and 69.38 ± 0.210% inhibition, respectively, at the highest concentration. The dichloromethane extract of *Z. officinale* (DCMEZO) exhibited the lowest scavenging activity among the plant extracts, with 49.44 ± 0.168% at 50 µg/mL. Ascorbic acid, used as a standard, showed the highest superoxide scavenging activity, with nearly complete inhibition at 98.45 ± 8.71% at the highest concentration tested.

Hydroxyl Radical Scavenging Activity

Table 3 and Figure 2 show that all plant extracts exhibited dose-

Table 1: Phytoconstituents of various extracts

Test	*B. serrate (n-hexane)*	*Z. officinale (Dichloromethane)*	*T. terrestris (Methanol)*	*C. sinensis (Hydroalcohol)*	*W. somnifera (Aqueous)*	*P. longum (Aqueous)*
Carbohydrates	-	-	-	+	+	+
Alkaloids	-	+	+	+	+	+
Glycosides	-	-	+	+	-	-
Tannins	-	-	-	+	-	-
Steroids	+	+	+	-	+	-
Triterpenoids	+	+			+	
Volatile oils	-	-	-	-	-	-
Fats and fixed oil	-	-	-	-	-	-
Flavonoids	-	+	+	+	+	-
Polyphenols	-	-	-	+	-	-
Saponins	-	-	+	+	+	-
Amino acids	-	-	-	+		-
Gums and mucilage	-	-	-	-	-	-

'+' Presence of constituents; '-'Absence of constituent

Table 2: Superoxide radical scavenging assay of various extracts

Plant extract (µg/mL)	*10*	*20*	*30*	*40*	*50*
NHEBS	34.07 ± 0.364	41.76 ± 0.208	52.59 ± 0.245	60.76 ± 0.309	69.63 ± 0.125
DCMEZO	27.74 ± 0.709	32.71 ± 0.276	38.49 ± 0.192	42.63 ± 0.298	49.44 ± 0.168
METT	33.067 ± 0.683	42.45 ± 0.241	54.25 ± 0.078	62.64 ± 0.205	69.90 ± 0.091
HAECS	35.80 ± 0.186	43.90 ± 0.338	58.52 ± 0.236	67.49 ± 0.194	73.79 ± 0.591
AEWS	33.59 ± 0.186	41.133 ± 0.045	53.23 ± 0.125	61.18 ± 0.036	69.38 ± 0.210
AEPL	34.23 ± 0.037	42.40 ± 0.115	56.31 ± 0.206	66.330 ± 0.065	71.53 ± 0.135
Ascorbic acid	63.45 ± 4.99	72.41 ± 5.12	82.18 ± 7.14	88.44 ± 9.31	98.45 ± 8.71

"NHEBS- n-Hexane extract of *B. serrate;* DCMEZO- Dichloromethane extract of *Z.r officinalis*; METT- Methanolic extract of *T. terrestris;* HAECS- Hydroalcoholic extract of *C. sinensis;* AEWS- Aqueous extract of *W. somnifera*; AEPL- Aqueous extract of *P. longum*"

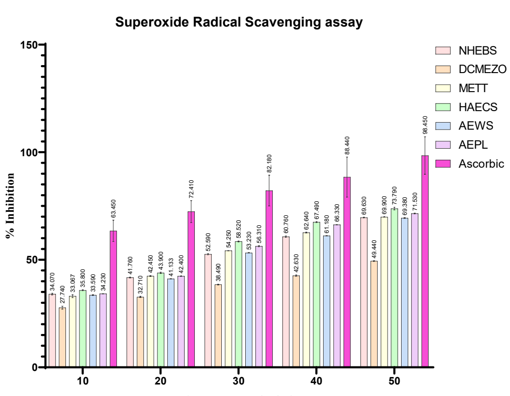

Figure 1: Superoxide radical scavenging assay of various herbal extracts

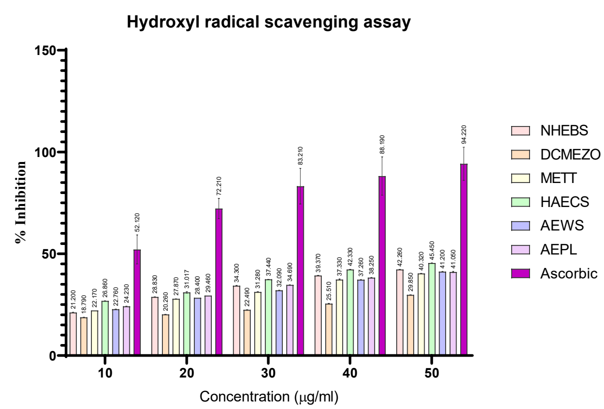

Figure 2: Hydroxyl radical scavenging assay of various herbal extracts

dependent scavenging activity. The hydroalcoholic extract of *C. sinensis* (HAECS) displayed the highest activity among the extracts, reaching 45.45 ± 0.047% inhibition at 50 µg/mL. This was followed by the aqueous extract of *W. somnifera* (AEWS) at 41.20 ± 0.053% and the methanol extract of *T. terrestris* (METT) at 40.32 ± 0.060%. The aqueous extract of *P. longum* (AEPL) showed moderate activity, achieving 41.05 ± 0.311%, while the n-hexane extract of *B. serrata* (NHEBS) demonstrated a maximum inhibition of 42.26 ± 0.045%. The dichloromethane extract of *Z. officinale* (DCMEZO) had the lowest hydroxyl radical scavenging activity, with 29.85 ± 0.032% inhibition at the highest concentration

Table 3: Hydroxyl radical scavenging assay of various herbal extracts

Plant Extract (µg/mL)	*10*	*20*	*30*	*40*	*50*
NHEBS	21.20 ± 0.119	28.83 ± 0.037	34.30 ± 0.110	39.37 ± 0.075	42.26 ± 0.045
DCMEZO	18.79 ± 0.052	20.26 ± 0.015	22.49 ± 0.012	25.51 ± 0.147	29.85 ± 0.032
METT	22.17 ± 0.025	27.87 ± 0.038	31.28 ± 0.093	37.33 ± 0.297	40.32 ± 0.060
HAECS	26.86 ± 0.042	31.017 ± 0.403	37.44 ± 0.024	42.33 ± 0.064	45.45 ± 0.047
AEWS	22.760 ± 0.036	28.40 ± 0.007	32.09 ± 0.033	37.26 ± 0.036	41.20 ± 0.053
AEPL	24.23 ± 0.034	29.46 ± 0.034	34.69 ± 0.108	38.25 ± 0.060	41.05 ± 0.311
Ascorbic acid	52.12 ± 7.11	72.21 ± 4.98	83.21 ± 8.77	88.19 ± 9.44	94.22 ± 8.12

"NHEBS- n-Hexane extract of *B. serrate;* DCMEZO- Dichloromethane extract of *Z. officinalis*; METT- Methanolic extract of *T. terrestris;* HAECS- Hydroalcoholic extract of *C. sinensis;* AEWS- Aqueous extract of *W. somnifera*; AEPL- Aqueous extract of *P. longum*"

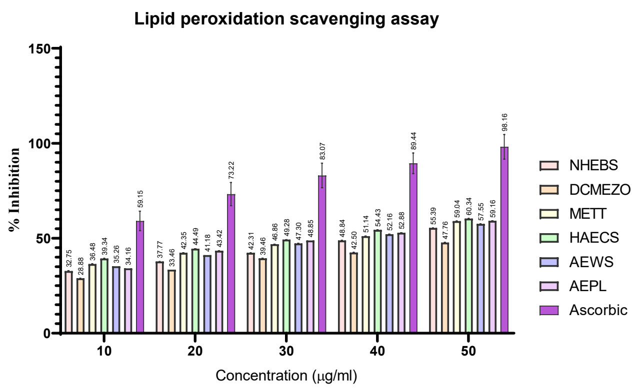

Figure 3: Lipid peroxidation scavenging assay of various herbal extracts

tested. Ascorbic acid, used as a standard, exhibited the highest scavenging activity, with 94.22 ± 8.12% inhibition at 50 µg/mL, significantly outperforming the plant extracts.

Lipid Peroxidation Scavenging Assay

The results of Table 4 and Figure 3 showed a dose-dependent increase in activity for all extracts tested. The hydroalcoholic extract of *C. sinensis* (HAECS) exhibited the highest activity, achieving 60.34 ± 0.075% inhibition at 50 µg/mL. This was closely followed by the methanol extract of *T. terrestris* (METT), which reached 59.04 ± 0.054%, and the aqueous extract of *P. longum* (AEPL) at 59.16 ± 0.027%. The aqueous extract of *W. somnifera* (AEWS) showed significant activity with 57.55 ± 0.074% inhibition. The n-hexane extract of *B. serrata* (NHEBS) and the dichloromethane extract of *Z. officinale* (DCMEZO) displayed the lowest activities among the extracts, with maximum inhibitions of 55.39 ± 0.009% and 47.76 ± 0.025%, respectively. Ascorbic acid, used as a standard, showed the highest lipid peroxidation scavenging activity, with 98.16 ± 6.45% inhibition at the highest concentration, significantly surpassing the activities of the plant extracts.

DISCUSSION

The study investigated the antioxidant properties and phytochemical profiles of plant extracts, including B. *serrata, Z. officinale, T. terrestris, C. sinensis, W. somnifera*, and *P. longum.* Historically, these plants have been used in herbal medicine, and their therapeutic potential is constantly being explored, especially in terms of their antioxidant properties. *C. sinensis* (green tea) hydroalcoholic extract showed the highest superoxide and hydroxyl radical scavenging activity, supporting previous findings indicating that green tea polyphenols have strong antioxidant properties.[11,12]

In general, *C. sinensis* powerful antioxidant properties are attributed to its high catechin content, particularly epigallocatechin gallate (EGCG), which is well-known for its health benefits.[13]

A methanol extract of *T. terrestris* also demonstrated significant antioxidant properties. Studies have shown *T. terrestris* contains significant antioxidant properties due to its saponins and polyphenols.[14] In addition, the plant is able to reduce inflammation and promote hepatoprotection, further highlighting its therapeutic value.[15]

Table 4: Lipid peroxidation scavenging assay of various herbal extracts

Plant Extract	*10*	*20*	*30*	*40*	*50*
NHEBS	32.75 ± 0.249	37.77 ± 0.037	42.31 ± 0.032	48.84 ± 0.040	55.39 ± 0.009
DCMEZO	28.88 ± 0.021	33.46 ± 0.030	39.46 ± 0.030	42.50 ± 0.032	47.76 ± 0.025
METT	36.48 ± 0.021	42.35 ± 0.072	46.86 ± 0.025	51.14 ± 0.075	59.04 ± 0.054
HAECS	39.34 ± 0.071	44.49 ± 0.049	49.28 ± 0.039	54.43 ± 0.046	60.34 ± 0.075
AEWS	35.26 ± 0.022	41.18 ± 0.003	47.30 ± 0.049	52.16 ± 0.025	57.55 ± 0.074
AEPL	34.16 ± 0.025	43.42 ± 0.101	48.85 ± 0.021	52.88 ± 0.043	59.16 ± 0.027
Ascorbic acid	59.15 ± 5.12	73.22 ± 6.14	83.07 ± 6.45	89.44 ± 5.41	98.16 ± 6.45

"NHEBS- n-Hexane extract of *B. serrate;* DCMEZO- Dichloromethane extract of *Z. officinalis*; METT- Methanolic extract of *T. terrestris;* HAECS- Hydroalcoholic extract of *C. sinensis;* AEWS- Aqueous extract of *W. somnifera*; AEPL- Aqueous extract of *P. longum*"

W. somnifera (Ashwagandha) was found to possess notable antioxidant properties, which support its traditional use in treating oxidative stress-related condition.[16,17] As a result of its bioactive compounds, such as withanolides, ashwagandha has both neuroprotective and anti-inflammatory properties, which makes it a valuable herb both in traditional and contemporary medicine.[18]

There was moderate antioxidant activity in the aqueous extract of *P. longum*. There are several bioactive compounds in *Piper* species, including piperine, which has antimicrobial, antioxidant, and anti-inflammatory properties.[19] Many traditional cultures utilize *P. longum* for its medicinal properties due to the presence of these compounds.[17]

In comparison with other extracts, *B. serrata* and *Z. officinale* showed lower antioxidant activity. It is important to note that both plants are known for their anti-inflammatory and medicinal properties. It is well known that *B. serrata* is used in the treatment of inflammation due to the presence of boswellic acids in it.[20] The ginger herb *Z. officinale* contains gingerols and shogaols, which are antioxidant and anti-inflammatory compounds.

CONCLUSION

There are significant antioxidant properties present in a number of plant extracts, including *C. sinensis, T. terrestris, W. somnifera*, and *P. longum*, with *C. sinensis* demonstrating the greatest potency. The results are in line with previous research indicating that these plants are effective in treating oxidative stress-related conditions. *Z. officinale* and *B. serrata* both exhibit lower antioxidant activities. A detailed investigation of these extracts in modern pharmacological applications is necessary to fully understand and utilize these compounds.

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