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Comparative analysis of phytochemical screening and antioxidant properties of three common medicinal plants

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Abstract
Methanolic extracts of three medicinal plant species, namely Hyptis suaveolens, Tridax procumbens, and Azadirachta indica, were screened for phytochemicals and antioxidant potential. DPPH (1, 1- diphenyl 2, picrylhydrazyl) radical, hydroxyl (OH.) radical, hydrogen peroxide (H2O2) radical, and superoxide (O2.) radical scavenging experiments were used to study antioxidant properties. Total phenolic content I (TPC) was measured using the Folin-Ciocalteu method, and total flavonoid content was measured using the aluminium chloride method (TFC). The methanolic extracts of A. indica had the highest radical scavenging ability, with an IC50 value of 11.71 2.01 g/ml in the DPPH experiment.

Keywords: phytochemical screening, antioxidant capacity, medicinal plants, IC50 value

Introduction

Medicinal plants have been utilised to cure human diseases for thousands of years because they contain a large and diverse range of chemical substances that can have a specific physiological effect on the human body [1]. Alkaloids, tannins, glycosides, steroids, flavonoids, terpenoids, saponins, and Phenolic are among the most important organic substances [2]. A variety of such compounds have been identified from plants and could be exploited to produce new medications to inhibit bacterial and fungal pathogen growth and quench reactive oxygen species (ROS) with novel modes of action and low toxicity to the host cell [3]. ROS, which include hydrogen peroxide (H2O2), hydroxyl radical (OH-), nitric oxide (NO-), and superoxide (O2-) anion, are potential degenerative agents in the human body [4, 5]. Due to oxidative damage to lipids, nucleic acids, and proteins, these free radicals are highly hazardous and are implicated in the aetiology of many chronic disorders [6].

Antioxidant chemicals are beneficial because they share electrons with free radicals. When a free radical acquires an electron from one of these molecules, it is no longer necessary to assault the cell, and the oxidation chain reaction is broken. Antioxidants have the ability to limit electron sharing without becoming reactive, hence they are not toxic in this condition [7]. Antioxidants can be natural or synthetic, however synthetic antioxidants like butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) are being phased out due to their hazardous and carcinogenic effects [8]. Fruits, seeds, bark, petals, leaves, roots, and stems, among other therapeutic plants, provide natural antioxidants. For its own defensive system against ROS, plants generate phenolic and flavonoid chemicals. Phenolic chemicals are not equally distributed throughout plants; they are concentrated in the outer layers of fruits, leaves, and barks [9]. Total phenolic content (TPC) and total flavonoid content (TFC) are used to determine antioxidant content, whereas DPPH, hydrogen peroxide, superoxide anion, and hydroxyl free radical scavenging ability are used to determine antioxidant characteristics.

The aromatic strongly scented herb Hyptis suaveolens Poit. (Lamiaceae), also known as "Ganga tulasi," is found in the Deccan Peninsula, North East India, Andaman and Nicobar Islands, Philippines, and tropical America. The herb is employed as a stimulant, carminative, for wounds, sudorific, galactogague, catarrhal condition, uterine infection, and parasitic skin illnesses in traditional medicine [10, 11].

The sunflower Tridax procumbens L. is a stunning member of the Asteraceae family. It is native to Central America and the West Indies, but it has spread across the tropics as a naturalised species. In several nations, it is used for a variety of indigenous medicinal purposes. Malaria, diabetes mellitus, sore throat, liver, and menstruation problems are all treated using decoctions of its various parts [12].

Azadirachta indica L., also known as 'neem,' is a very versatile medicinal plant with a wide range of biological activities. Most tropical and subtropical nations have naturalised it. It is a broad-leaved evergreen that grows up to 30 metres tall and belongs to the Meliaceae family of mahogany trees. Because each portion of A. indica's leaves, bark, stem, or root has therapeutic benefits, it has been widely employed in Ayurveda, Unani, and homoeopathic medicine [13].

As a result, knowledge of medicinal plants and their medical benefits is critical for reducing health problems and preserving data for future research. As a result, the primary goals of this study are to I investigate the qualitative and quantitative analysis of three distinct medical plants, and (ii) investigate the antioxidant capacity of three

different medicinal plants. The antioxidant effects of extracts from such plants are receiving a lot of attention in the food and pharmaceutical industries.

Materials and Methods

1. Plant Material Collection and Processing

The plant samples were obtained in February 2018 from Berhampur, Odisha, India (between 190 13' 48.948"N and 190 23'59.046" N latitudes and 84 °43'58.877"E and 85 °04'16.707"E). The dirt was removed from the selected plant portions, which were then washed under flowing tap water. After a few days of oven drying at 60°C, the plant samples were crushed into fine powder and kept in polythene bags for later use.

2. Plant Extract Preparation

Using the Soxhlet equipment method [14], measured plant components were extracted with methanol. Finally, all extracts were evaporated in a water bath to yield the corresponding extracts, which were then stored at -200C until further analysis [15, 16]. The following formula was used to calculate the percent of yield [17]:

$$\text{Yield } \% = (M_2 - M_1)/M_0 \times 100$$

Where M2 represents the weight of the extract plus the container, M1 represents the weight of the container alone, and M0 represents the weight of the initial dried sample.

3. Reagents and Chemicals

All of the chemicals and reagents used in the study were of standard quality. Fehling's solution A and B, Mayer's reagent, Iodine solution Sodium carbonate, rutin, DPPH (1,1-Diphenyl 2-Picrylhydrazyl), Ascorbic acid, FeSO4, Hydrogen Peroxide (H2O2), Sodium salicylate, Nitro Blue Tetrazolium (NBT), Nicotinamide Adenine Dinucleotide (NADH), Phenazine Methosulfate (PMS), Tris HCl buffer, Phosphate buffer, Sodium.

4. Phytochemicals Qualitative Analysis

Using standard qualitative techniques, qualitative studies of methanolic extracts were performed to detect the presence of several bioactive chemicals [18].

5. Properties of Antioxidants

The Folin- Ciocalteu technique was used to quantify the content of TPC in plant extracts with minor modifications [19, 20]. In a test tube, pour 20 l of methanolic extract (1 mg/ml) and 1.58 ml of distilled water, then add 7% of 100 l of Folin-reagent. Ciocalteu's After waiting 8 minutes, 10% of a 300 l saturated sodium carbonate solution (250 g/l) was added. The absorbance was measured in triplicate at 765 nm after the solution had been incubated for 2 hours at room temperature. The standard curve was calibrated with gallic acid (0-500 mg/l). The results were represented in milligrammes of gallic acid equivalent per gramme of dry plant material (mg GA/g).

Spectrophotometric technique was used to calculate the TFC [21, 20]. 1 ml of a 1 mg/ml methanol solution of the extract and 1 ml of a 2 percent AlCl3 solution dissolved in methanol made up the crude extract. At room temperature, the solutions were incubated for an hour. In triplicate, the absorbance was measured at 415 nm. The standard curve was developed using the same technique as for the rutin standard solution. The flavonoid content of the extract was then converted to rutin equivalent (mg of RE/ g) dry weight of plant material.

6. DPPH Free Radical Scavenging Assay

The DPPH test [20] was used to determine the methanolic extracts' free radical scavenging capability. In methanol, a DPPH solution (0.004% w/v) was produced. Using methanol, a stock solution of methanolic extract of plant (1 mg/ml) and standard ascorbic acid (0.05 mg/ml) were made. In test tubes, various quantities (10-500 g/mL) of the plant extract and ascorbic acid were added, along with 1 mL freshly made DPPH solution. The test tubes were then covered with aluminium foil to shield them from light. Each test tube was filled to a final volume of 2ml with methanol and incubated at room temperature for 30 minutes in the dark. A spectrophotometer was used to measure the absorbance after incubation at 517 nm.

A control sample was made with the same amount of methanol and DPPH as the test sample but no extract or reference ascorbic acid. Methanol was used as a control. The following formula was used to determine radical scavenging:

$$[(\text{Abs of control} - \text{Abs of sample})/ \text{Abs of control}] \times 100 = \text{percent inhibition.}$$

7. Hydroxyl Radical Scavenging Assay

The extracts (10-500g/mL) were added to a reaction mixture (3 mL) comprising 1 mL FeSO4 (1.5 mM), 0.7 mL hydrogen peroxide (6 mM), 10% of 0.3 mL sodium salicylate (20 mM), and varied quantities of the extracts (10-500g/mL). The absorbance of the hydroxylated salicylated complex was measured at 562 nm after 1 hour of incubation at 370C [20]. The standard utilised was ascorbic acid. The percentage scavenging effect was determined as [1- (A1 – A2)/ A0] 100, where A0 was the absorbance of the control (without extract), A1 was the

absorbance in the presence of the sodium salicylate extract, and A2 was the absorbance without sodium salicylate extract.

8. Superoxide Anion Radical Scavenging Assay

Under aerobic conditions, nitro blue tetrazolium (NBT) was reduced in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) [20]. 1 mL NBT (50 M) solution, 1 mL NADH (78 M) solution, and a sample solution of extract (10-500g/mL) in distilled water mixed in Tris HCl buffer (3mL, 16 mM, pH 8.0). 1 mL of PMS solution (10 M) was added to the mixture to start the reaction. The reaction mixture was incubated for 5 minutes at 250°C, and the absorbance was measured at 560 nm in comparison to the blank samples. As a control, ascorbic acid was employed. The reaction mixture's lower absorbance suggested improved superoxide anion scavenging activity.

9. Hydrogen Peroxide Radical Scavenging Assay

The extract's ability to scavenge hydrogen peroxide (H2O2) was calculated using the method of [20]. In phosphate buffer, pH 7.4, a 40 mM hydrogen peroxide solution was produced. A UV-visible spectrophotometer was used to assess the quantity of hydrogen peroxide by measuring absorbance at 230 nm. After 10 minutes, the extracts (10-500g/mL) in distilled water were added to a hydrogen peroxide solution at 230 nm and compared to a blank solution containing phosphate buffer but no hydrogen peroxide. As a control, ascorbic acid was employed.

10. Analytical statistics

All of the studies were done in triplicate, and the findings are shown as mean std dev. The IC50 value was calculated using linear regression analysis.

Results and Discussion

The yield percentages of the methanolic extracts of H. suaveoelnsis, T. procumbens, and A. indica were 19.72 percent, 22.32 percent, and 23.68 percent, respectively, after the plants were extracted.

1. Phytochemical screening

The findings of a phytochemical screening test of three distinct medicinal plant leaf extracts are shown in Table 1. All three plants' extracts included alkaloids. In the meantime, terpenoids, protein, and coumarin have been discovered in H. A. suaveolens and suaveolens indica. Other secondary metabolites found in T included phenol, tannin, steroids, and leucoanthocyanin. A. procumbens and procumbens indica. Only A contained reducing sugar, saponins, and glycosides. indica. Anthocyanin, on the other hand, was only found in T. procumbens. These bioactive components, along with others found naturally in most plant materials, are known to be bactericidal, pesticidal, or fungicidal in nature, giving plants antimicrobial properties [22, 23, 24].

Table 1: Phytochemical screening of methanolic extracts three medicinal plants.

Sl no	Phytochemical test	*H. suaveolens*	*T. procumbens*	*A. indica*
01	Alkaloids	+	+	+
02	Terpenoids	+	-	+
03	Phenol and Tannin	-	+	+
04	Reducing sugar	-	-	+
05	Sapnonin	-	-	+
06	Protein	+	-	+
07	Steroid	-	+	+
08	Anthocyanin	-	+	-
09	Coumarin	+	-	+
10	Leucoanthocyanin	-	+	+
11	Glycosides	-	-	+

2. Antioxidant Properties

The total phenolic content of the crude extracted from the leaves of H. suaveolens, T. procumbens, and A. indica was determined using the Folin Ciocalteu reagent. A. indica methanolic extract had the highest total phenolic content, while H. suaveolens methanolic extract had the lowest. Total phenolics were measured in the following order: A. indica > T. procumbens >H. suaveolens, with values of 15.01 0.002, 12.18 0.001, and 10.22 0.001 GAE mg/g, respectively. A spectrophotometric approach was used to quantify the total flavonoid content of medicinal plant extracts. T. procumbens had the highest concentration of total flavonoids, while A. indica had the lowest.

T. procumens > A. indica >H. suaveolens had the highest total flavonoids content, with values of 13.42 0.003, 11.34 0.003, and 7.46 0.001 respectively (Table 2). The presence of phenols in the plant suggests that it may have therapeutic properties. Some phenolic compounds are thought to be cancer preventives [25]; these are chemicals that may reduce the likelihood of developing cancer [26], which is estimated to be the leading cause of death worldwide and also plays a role in the development of cardiovascular disorders [27]. Phenolic chemicals contained in plants may also act as antioxidants by interacting with and scavenging highly reactive substances

before they can react with other biomolecules and cause serious damage. Flavonoids have long been known for their antibacterial properties, but they've lately been discovered to have immunoregulatory properties [28]. Tannins have also been linked to antiviral, antibacterial, and antiparasitic properties [29].

Table 2: Total Phenolic and flavonoids content of three medicinal plant extracts.

Plant extract	Total Phenolic content mg/g, plant extract (in GAE)	Total Flavonoid content mg/g, plant extract (in RE)
H. suaveolens	10.22 ± 0.001	7.46 ± 0.001
T. procumbens	12.18 ± 0.001	13.42 ± 0.003
Indica	15.01 ± 0.002	11.34 ± 0.003

DPPH is a stable free radical that accepts an electron or hydrogen radical to produce a stable diamagnetic molecule, which is commonly employed for studying the scavenging activity of chemicals. The antioxidant changes the dark blue (deep violet colour) of the DPPH radical solution to a lighter (yellow) colored,-diphenyl—picryl hydrazine; thus, the discoloration indicates the antioxidant compound's or extracts' radical scavenging potential activity in terms of hydrogen or electron-donating ability. This assay is extremely beneficial in the search for a stable antioxidant medicine that may lower the amount of free radicals produced [30, 31, 32, 33]. The DPPH free radical scavenging activity of three distinct plant extracts was tested (Fig 1). At 500 g/mL, A. indica had the highest radical scavenging activity (84.28%), followed by T. procumbens (77.12%), and H. suaveolens (75.12%). (75.28 percent). The scavenging activity was compared to conventional ascorbic acid, which has a maximum scavenging activity of 94.92 percent at 500 g/mL. At 500 g/mL, A. indica had the highest hydroxyl radical scavenging activity (70.85 percent), followed by T. procumbens (56.53 percent), and H. suaveolens (52.67 percent) (Fig 2). Standard ascorbic acid has a maximal scavenging activity of 71.88 percent at 500 g/mL. The hydroxyl radical reacts with nucleotides in DNA to generate strand breakage, which leads to carcinogenesis, mutagenesis, and cytotoxicity, according to Khan *et al*. [34]. Figure 3 depicts the hydrogen peroxide scavenging activity of methanolic leaf extracts from three plants and a standard. At 500 g/mL, T. procumbens had the highest scavenging activity (79.45%), followed by H. suaveolens (74.40%) and A. indica (67.76%). At 500 g/mL, H. suaveolens had the best scavenging activity (56.62 percent), followed by A. indica (55.77 percent), and T. procumbens (48.02 percent) in the super oxide assay (Fig 4). A variety of biological processes, including enzyme systems including lipooxygenase, peroxidase, NADPH oxidase, and xanthainoxidase, create superoxide anion radicals. Superoxide anions are involved in the generation of additional cell-damaging free radicals and play a significant function in plant tissue [35]. Superoxides are also recognised for indirectly initiating lipid peroxidation as a result of H2O2 production, producing hydroxyl radical precursors [36]. Plants' hydroxyl groups are responsible for H2O2 components and their ability to scavenge radicals [37]. Because of their possible antioxidant activity, plant phenolics found in fruits and vegetables have gotten a lot of attention [38].

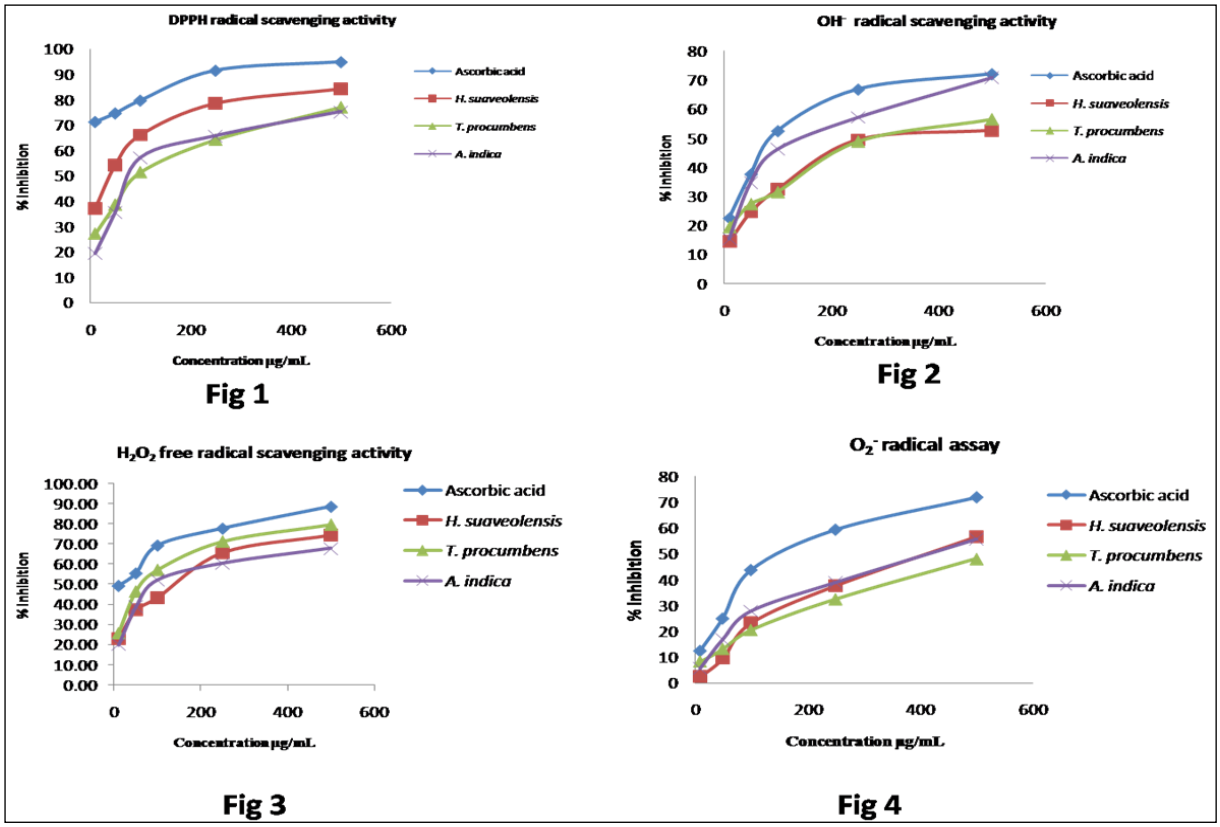

Fig 1-4: Antioxidant potential of methanolic extracts of three medicinal plants.

3. IC50 Value

Table 3 shows the inhibitory concentrations (IC50) of three different plant species' methanolic extracts. It's worth noting that the IC50 value with the lowest value indicates the highest activity against free radicals. In comparison to hydrogen peroxide, hydroxyl radical, and superoxide anion radical, the findings of the trials revealed that DPPH trapped the most free radicals. The scavenging activity of the studied extracts was varied in the DPPH assay, with A. indica (IC50 = 11.71 ± 2.01 µg/mL) showing the highest activity against free radicals, followed by T. procumbens (16.94 ± 2.60 µg/mL) and H. suaveolens (25.17 ± 0.13 µg/mL). Standard Ascorbic acid had an IC50 value of 4.89 ± 3.04 µg/mL. The hydroxyl radical (OH-) effect's scavenging activity rises with the concentration of the standard and sample. The activity of A. indica (IC50 = 62.18 ± 3.07 µg/mL) was lower than that of normal ascorbic acid (59.55 ± 3.60 g/mL). In hydrogen peroxide, ascorbic acid had a larger proportion of free radical inhibition than the other extracts, with an IC50 of 55.86 ± 4.84 g/mL. In the current investigation, the superoxide anion assay revealed that ascorbic acid had the strongest anti-free radical action when compared to other plant extracts, with an IC50 = 48.92 ± 3.59 µg/mL.

Table 3: IC50 values of the free radical scavenging activities of the methanolic extracts of three plant species.

IC50 value (µg/mL)				
Parameters	**Ascorbic acid**	***H. suaveolens***	***T. procumbens***	***A. indica***
DPPH radical assay	4.89 ± 3.04	25.17 ± 0.13	16.94 ± 2.60	11.71 ± 2.01
OH^- radical assay	59.55 ± 3.60	88.37 ± 3.04	76.89 ± 3.78	62.18 ± 3.07
H_2O_2 radical assay	55. 86 ± 4.84	95.71 ± 3.98	118.27 ± 2.39	61.12 ± 4.22
O_2^- radical assay	48.92 ± 3.59	61.21 ± 3.59	66. 33 ± 5.02	50.28 ± 3.59

Conclusion

As our understanding of the processes of human diseases has grown, particularly metabolic disorders like diabetes, liver disease, and hypertension, the role of highly reactive oxygen species like free radicals has become increasingly essential. Natural antioxidant research on medicinal plants is also on the rise. According to the findings, the leaves of three different plants have antioxidant characteristics and may scavenge free radicals. There was a significant link discovered between antioxidant capabilities and overall Phenolic content. As a result, additional research is required to isolate and identify the active components in the plant extract.

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Conflict of Interest

There is no conflict of interest, we said.

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None

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