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Article

Phytochemical Analysis of *Amaranthus spinosus* Linn.: An *in vitro* Analysis

Ishir Sharma[1], Pooja Kasture[2], Ankita Umrao[2], Jyothsna Rao[2], Gururaj Rao[2]
[1] The International School Bangalore, NAFL Valley, Whitefield – Sarjapur Road, Bangalore, KA, India, 562125
[2]iCREST-International Stem Cell Services Limited, 9/1, Mission Road, Bangalore

SUMMARY

Mainstream cancer treatments, which include radiotherapy and chemotherapeutic drugs, are known to induce oxidative damage to healthy somatic cells due to the liberation of harmful free radicals. In order to avert this, physiological antioxidants must be complemented with external antioxidants. We performed a preliminary phytochemical screen to identify alkaloids, saponins, flavonoids, polyphenols, and tannins in all parts of the *Amaranthus spinosus* Linn. plant. Based on the hits we obtained, we combined the methanolic extract of the leaves with the acetonic extract of the inflorescence, which resulted in a highly potent formulation which was abundant with the all the desired phytocomponents such as saponins, tannins, polyphenols, flavonoids, alkaloids. We hypothesised that this crude herbal extract, AS20, will exhibit high antioxidant properties. Using the 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) assay, we found that the half maximal inhibitory concentration (IC50) value of this formulation was 85.27 µg/mL. This paper describes the preparation of this crude extract and assesses its antioxidant properties for potential use in complementary cancer treatment.

INTRODUCTION

Integrative oncology is a rapidly emerging branch of the health sciences which aims to complement the existing course of cancer treatment with other remedies to avert the side effects of mainstream treatment (1). Examples of mainstream cancer treatments include surgery, chemotherapy, and radiotherapy. Oncologists worldwide are recognizing the need to prevent the deleterious side effects that such treatments induce upon patients. In an effort to prevent oxidative damage of cells by harmful free radicals, such as reactive oxygen species (ROS) (2), oncologists have embraced the usage of herbal antioxidants alongside the course of cancer treatment. Hence, by complementing physiological antioxidants with external herbal products, we would theoretically be able to prevent oxidative cell damage to healthy body cells, while retaining the anticarcinogenic properties of the mainstream drug. This would remove the need for alternative methods of treatments, as such methods integrate into pre-existing therapies. Evidently, the scope for research in this field is vast, as researchers search for more methods such as this one to administering cancer drugs more efficiently. In this paper, we will assess the ability of a crude herbal extract of the *Amaranthus spinosus* (AS) plant to exhibit significant antioxidant properties. To create this herbal formulation, we combined extracts of different parts of the plant which had the highest concentration of the desired phytocomponents, based on the preliminary phytochemical analysis of the plant. This paper describes the preparation of a crude extract which is a true representative of the potency of the entire *Amaranthus spinosus* (AS) plant.

The *Amaranthus spinosus* Linn. plant ('Spiny amaranth', 'Prickly amaranth', or 'Spiny pigweed') (3) is a medicinal plant which is found in abundance in India and around the world. This plant is commonly found as a weed in rice agriculture (4). Historically, this plant has been used extensively in Ayurveda (5), the traditional folk medicine of India, to treat diabetes, jaundice (6), and various other ailments. Previous studies have shown this plant to possess several medicinal properties, including anti-inflammatory (7), anti-diabetic (8), anti-diarrheal (9), anti-bacterial (10), anti- microbial (11), and anti-malarial (12) properties. Hence, the medicinal properties of this plant are of keen interest to the scientific community. While previous phytochemical studies have been done on individual parts of this plant, such as the leaves, the properties of the entire plant are relatively unknown to the world of science, and there has been little attempt to prepare pharmaceutical drugs from this plant. The most common way to prepare a pharmaceutical drug is to isolate a specific phytocomponent. However, given our goal to create an herbal crude extract, the total content of the desired secondary metabolites was determined for each part of the plant (leaves, root, stem, inflorescence).

The normal course of treatment for cancer is to employ surgery, chemotherapeutic drugs, or radiotherapy. While the usage of radiation or drugs does kill the cancer cells, it also induces cell stress, which causes an increase of harmful free radicals, such as reactive oxygen species (ROS), which react with various cell parts, including the phospholipid bilayer, amino acids, and DNA, causing severe oxidative damage to all these cell components (13). Furthermore, physiological antioxidant enzymes, such as superoxide dismutase, are inhibited by the activity of anti-cancer drugs. As a result, it becomes a necessity to include an external antioxidant dosage to complement the existing therapies in order to avert the side effects on healthy somatic cells.

Secondary metabolites are organic compounds produced primarily by plants as waste products of their metabolism.

They exhibit significant pharmacological and biological properties and are hence used for medical purposes (14). The secondary metabolite groups considered in this study were alkaloids, saponins, polyphenols, flavonoids, and tannins. The total content of each of these phytochemicals is usually determined by carrying out phytochemical tests (based on colorimetry) for each of these secondary metabolites. Alkaloids are basic compounds with heterocyclic rings. Their most common functional group is the amine group (NH2). The nitrogen acts as a Brønsted base, its lone pair of electrons acting as proton acceptors, and the hydrogen atoms act as proton donors (or a Brønsted acid) for hydrogen bonding. The acid-base properties of the amine group make alkaloids ideal bioactive molecules as they have the ability to bind to different proteins, receptors, and drug targets (15). Alkaloids have been shown to display significant antioxidant properties (16). Saponins are glycosidic compounds whose amphipathic nature gives them the ability to interact with various cell membrane components, such as cholesterol and phospholipids. Saponins are reported to be effective *in vitro* antioxidants (17). Polyphenols are polymers of phenols, which consist of a six membered cyclical ring (phenyl group). Polyphenols are known to have considerable antioxidant properties, which have been extensively studied over the last few decades (18). Their bioactivity comes from their ability to form hydrophobic and ionic bonds with proteins (19). Flavonoids are a type of phenolic compound which also possess antioxidant properties *in vitro* (20). They are abundantly present in tea and other plant-derived beverages. Tannins are water soluble phenols which are known to be proton donors. Their antioxidant properties include free radical scavenging, inhibition of stages of oxidation, and chelation of metal cations (21).Thus, we hypothesised that the crude extract, containing a greater content of these secondary metabolites compared to solvent extractions of individual parts of the plant, would display significant free radical quenching properties, as determined through calculation of the half maximal inhibitory concentration (IC50) value for the crude extract formulation obtained using the 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) assay.

RESULTS

The phytochemical screening of the AS plant extracts consisted of a series of chemical tests, based on colorimetry, which yielded the total concentration of each of the secondary metabolites in each solvent extraction. We measured these using Dragendorff's test for alkaloids, vanillin-sulphuric acid for saponins, aluminium chloride colorimetric assay for flavonoids, Folin-Ciocalteu's (FC) reagent for phenolic content, and the FC method for tannins. We prepared extracts of the individual parts of the plant using a range of solvents and phytochemically analysed them to determine the total content of the secondary metabolite groups of interest. We then prepared a formulation by combining the extracts with the highest concentration of anti-oxidants. A DPPH assay was then carried out on this formulation to quantify the amount of antioxidants present in the extract.

Solvent	Sample	Saponin test	Polyphenol test	Flavonoid test	Alkaloid test	Tannin test
Water Extract	Root	+++	+	+	++	+
	Inflorescence	+++	++	+	++	-
	Stem	+++	+	+	++	-
	Leaf	+++	+++	++	+++	-
Methanol Extract	Root	+++	+	-	-	-
	Inflorescence	+++	++	-	++	+
	Stem	+++	+	-	-	-
	Leaf	+++	+++	-	+++	-
Ethanol Extract	Root	+	-	+	-	-
	Inflorescence	+	+	-	++	+
	Stem	+	+	+	-	-
	Leaf	+	-	-	++	+
Chloroform Extract	Root	-	+	-	-	-
	Inflorescence	+	+	-	++	+
	Stem	-	-	-	++	+
	Leaf	-	-	-	-	-
Acetone Extract	Root	-	-	+	-	-
	Inflorescence	-	-	+++	-	-
	Stem	+	-	+	-	-
	Leaf	-	-	+	++	+

Table 1: The qualitative analysiswere tabulated using +++ ,++,+ for presence of phytochemicals and – for absence of phytochemicals and quantitative ranges of concentration were tabulated using the following notation. +++ for 1001-2000µg/ml, ++ for 501-1000 µg/ml,+ for 0-500 µg/ml.

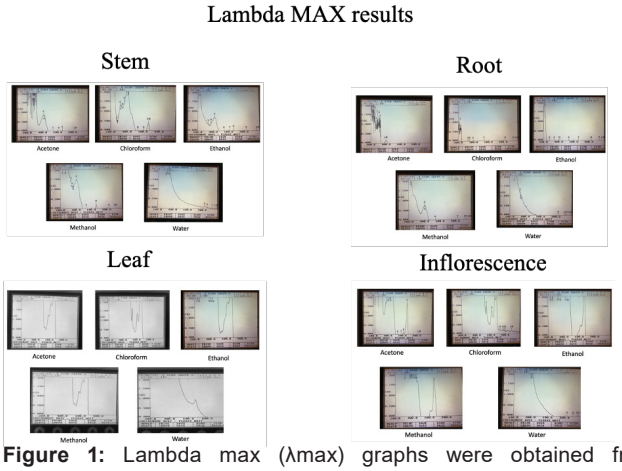

Figure 1: Lambda max (λmax) graphs were obtained from the spectrophotometer. The peaks represent the presence of phytocomponents. Spectra were measured at a wavelength range from 200-1000 nm to validate the presence of phytocomponents, prior to the screening of the different plant parts.

From the lambda max readings **(Figure 1)** and the phytochemical screening results **(Table 2)**, it was evident that certain solvent extracts of particular parts of the plant contained high amounts of all the phytocomponents **(Figure 2)**. The primary aim of the phytochemical screening was to determine the parts of the plant which contained the highest content of specific desired phytochemicals. Therefore, AS20 was prepared according to the following formulation, which was representative of the maximum potency of the entire plant:

AS20 = Inflorescence Acetonic extract + Leaf Methanolic extract

The methanolic extract of the leaves displayed a substantially high presence (+++) of nearly all the phytochemical groups, apart from flavonoids and tannins **(Table 2)**. Another group of interest was the inflorescence, which was the only group which displayed high presence (+++) of flavonoids (which are known to possess antioxidant properties) (22). As a result, the acetonic extract of the inflorescence and the methanolic extract of the leaves of the AS plant were combined to produce a drug (AS20) which had a high presence (+++) for all the phytochemical groups considered **(Table 2)**. Furthermore, AS20 displayed free radical scavenging property, as indicated by the DPPH assay results **(Figure 3)**. The % inhibition of free radicals increased linearly with concentration of the formulation, represented by the equation y=0.5281x+4.9671 (R2 ~ 0.98). From the equation obtained, the IC50 value (concentration of the drug at which 50% of free radical inhibition occurs) was calculated as below:

$$(50) = 0.521x + 4.9671 \rightarrow \therefore x = \frac{50 - 4.9671}{0.521} = 85.27\mu g\ mL^{-1}$$

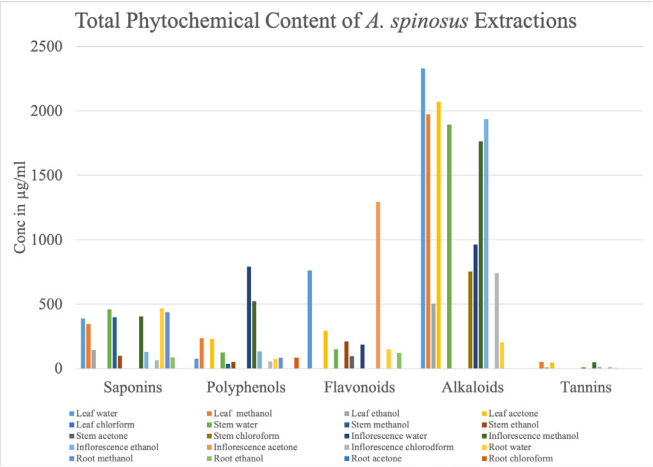

Figure 2: Bar graphs displaying total content of each phytochemical for all extractions (water, methanol, ethanol, acetone, and chloroform) of A. spinosus leaf, stem, inflorescence, and root. The concentration readings were measured in µg/mL. The quantitative phytochemical studies were based on the data in Table 2.

DISCUSSION

The rationale behind the preparation of AS20 was to combine specific solvent extracts from various parts of the AS Linn. plant with the greatest concentrations of all the phytochemical groups (alkaloids, saponins, tannins, flavonoids, and polyphenols). The result was the herbal combination AS20, which contained all the studied phytochemicals in high concentrations. Prior studies of the entire plant have assessed the antioxidant properties of the entire plant in hydroalcoholic extracts, wherein the IC50 value obtained by the DPPH method was 525.593 µg/mL (23). Moreover, the IC50 value for the DPPH assay of the methanolic extract of the leaves of AS as determined by Kumar et al. (24) was 87.527 µg/mL. Having obtained a marginally lower IC50 value of 85.27 µg/mL in this study, we have shown that AS20 also may have a mildly higher antioxidant capacity than both its individual constituents as well as the generic extract of the entire plant. The AS20 formulation is thus one which is representative of a higher antioxidant potency of the AS plant relative to its individual constituents. The antioxidant property of the formulation thus has implications for usage as a complementary dosage during cancer treatment to assist in averting damage to healthy somatic cells due to harmful free radicals produced during treatment.

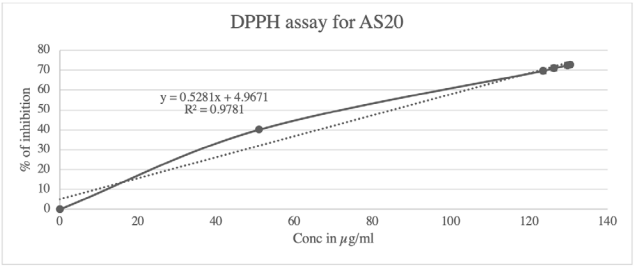

Figure 3: Line graph for DPPH assay results. The graph depicts the % inhibition of free radicals with increase in concentration of AS20 in µg/mL. This graph is representative of the antioxidant property of the formulation in terms of free-radical scavenging capacity. The IC50 value was determined using the graph.

Due to the many steps involved, we had potential for experimental errors, such as pipetting errors. Specifically, we assumed that for steps performed at room temperature, it stayed constant over the course of the experiment. We did not thermostatically monitor the laboratory temperature. We assumed the start time for incubation of all the concentrations to be the same for the DPPH assay, whereas we added the reagent sequentially into each of the five test tubes.

A limitation of our study is that we assessed the free radical scavenging ability of the formulation but not that of the extracts of the different parts of the plant, for which we relied on literature. Furthermore, we conducted the qualitative and quantitative phytochemical studies once for each extract. Future studies could 1) compare the antioxidant properties of the formulation with those of its individual components to confirm that it has a higher overall antioxidant potential than each of its components, 2) increase the number of trials and calculate the average phytochemical content of each extract to improve the reliability of the results, and 3) explore employing other antioxidant assays, such as the Total Antioxidant Capacity (TAC) Assay.

Future explorations could include expanding the scope of investigation to include examining this formulation for its other pharmacological activities, such as anticancer, antimicrobial, and antifungal activities. This will permit the assessment of other properties which may be beneficial in treatment of various ailments, such as cancer. In addition, other species of the Amaranth genus could be assessed. The efficacy of crude extracts from other species can be compared to the AS20 formulation. Furthermore, inter-species combinations

may also be explored for enhanced biological activities.

MATERIALS AND METHODS

Extract preparation

A. spinosus plants were obtained from a local nursery in Bengaluru. The leaves, stem, and inflorescence of the plant, as shown in **Figure 4**, were separated using scissors and cleaned by hand to remove insects. Subsequently, they were spread out over a large cloth and air-dried for a period of five days, to ensure complete removal of moisture content. After drying, the samples were ground separately in a grinding mixer and sieved into separate centrifuge tubes. The solvents used for extraction (water, methanol, ethanol, chloroform, and acetone) were selected to have a range of polarities.

The process of ground-drying using a grinding mixer was not feasible for the AS roots due to their fibrous nature. Hence, the process of maceration was employed wherein 1 g of roots were soaked in 20 mL of each solvent at room temperature for 24 hours. For each plant part, 1 g of ground powder was measured using a weighing balance and was dissolved in 10 mL of each of the five solvents [1 g/10 mL]. The contents were then decanted out of the conical flasks into centrifuge tubes. The tubes were centrifuged for 15 minutes at 3000 rpm to remove undissolved debris. Subsequently, the supernatants were collected into clean test tubes labelled according to plant part and solvent. A 1:2 dilution of crude sample volume to solvent volume ratio was then carried out in these tubes using the respective solvents. The centrifuge tubes containing leaf, stem, inflorescence, and root extract were then stored at 5°C.

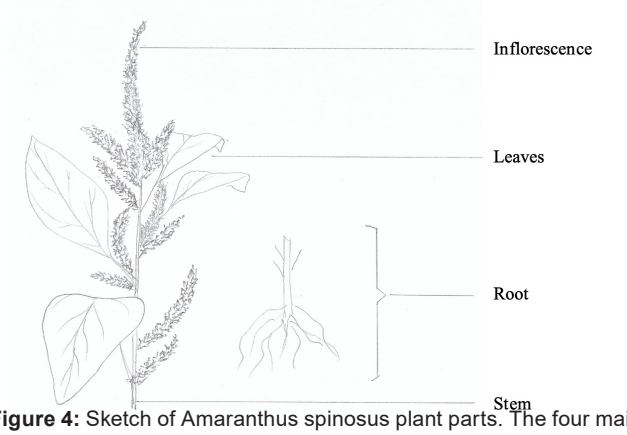

Figure 4: Sketch of Amaranthus spinosus plant parts. The four main parts of the plant are labelled as inflorescence, leaves, root, and stem. These parts were separated by hand prior to extraction.

Phytochemical Screening

Lambda max (λ_{max}) is the wavelength at which a compound displays its highest absorption in the spectrophotometer. The lambda max value represents a peak, which indicates the presence of phytocomponents **(Figure 1)**. Phytochemical screening involved the analysis of the total content (concentration) of alkaloids, saponins, flavonoids, polyphenols, and tannins.

Preliminary Qualitative Analysis:

Qualitative analyses were carried out according to the methods used by Kokate (25) and Rahman Gul et al. (26) **(Table 2)**. In the Dragendorff's test for alkaloids, 0.2 mL of diluted extract was added to 0.5 mL of each extract and 1 mL of Dragendorff's reagent was then added to this solution. An orange-brown precipitate was formed if alkaloids were present. In the olive oil test for saponins, 0.5 mL of each extract was added to 0.2 mL of olive oil, shaken vigorously, and left standing for 5 minutes. A soluble emulsion was formed if saponins were present. In the alkaline reagent test for flavonoids, five drops of 5% NaOH were added to 0.5 mL of extract, which yielded a yellow colour, then a few drops of 2 molar HCl were added to the solution. The solution became colourless if flavonoids were present. For polyphenols, the Folin-Ciocalteu's (FC) reagent test was carried out. Four drops of FC reagent and Na2Co3 were added to 0.5 mL of the sample, which was incubated at room temperature in the dark for 20 minutes. A blue colour was formed if polyphenols were present. For tannins, a ferric chloride test was carried out. A few drops of 5% FeCl3were added to 0.5 mL of extract and a blue-green colour formed if tannins were present.

Quantitative Analysis:

Quantitative analysis was performed as follows **(Table 2)**:

Phytocomponent	Qualitative Test	Quantitative Analysis	Standards used for Quantitative Analysis
Alkaloid	Dragendorff'sTest	Total Alkaloids	Bismuth Nitrate
Saponin	Olive Oil Test	Total Saponins	Diosgenin
Flavonoid	Alkaline Reagent Test	Total Flavonoids	Quercetin
Polyphenol	Folin-Ciocalteu's Test	Total Polyphenols	Gallic Acid
Tannin	Ferric Chloride Test	Total Tannins	Tannic Acid

Table 2: Phytochemical tests and standards. The qualitative and quantitative tests for alkaloids, saponins, flavonoids, polyphenols, and tannins were tabulated, along with the respective standard solutions used for quantitative analysis.

Total Alkaloid Content

To determine the total alkaloid content, the procedure used was adapted from that followed by Ellul (27). The chemicals used were 50 mg/L bismuth nitrate, 1 M thiourea, 1 M diluted hydrochloric acid, and 5% disodium sulphide. The standard curve was obtained with bismuth nitrate pentahydrate stock solution. Serial dilutions of standard stock was made by pipetting out 0, 0.2, 0.4, 0.6, 0.8 and 1 mL respectively. Standard stock of 50 mg/L Bismath nitrate pentahydrate was taken in the test tubes with 0.2 mL of test samples. Five millilitres of thiourea solution was added, and water was used to make up the volume to 10 mL. Absorbance was read at 435 nm. For the herbal sample, the samples were maintained at pH 2-2.5 with 1 M hydrochloric acid. Subsequently, 2 mL of Dragendorff's solution was added, and the solution was centrifuged for 10 minutes at 1500 rpm for precipitation. After centrifugation, 2 mL of disodium sulphide solution was added, and a brown-black precipitate was formed. Two millilitres of concentrated nitric acid was then added, and the volume was made up to 10 mL with water. One millilitre of solution was

discarded, and 5 mL of thiourea was added. The absorbance was read, and the concentration was determined on the basis of the standard curve.

Total Saponin Content

To determine the total saponin content, the vanillin-sulphuric acid method (28) was used. The chemicals used were 8% (w/v) vanillin, 72% (v/v) sulphuric acid, and 0.5 mg/mL diosgenin. A quantity of 0.25 mL of sample was added to 0.25 mL of 8% vanillin in ethanol and 2.5 mL of 72% sulphuric acid. The solutions were incubated for 15 minutes at 60°C. The absorbance of the standards were measured at 765 nm. A standard curve was plotted against diosgenin, and the concentration was hence determined by extrapolation.

Total Flavonoid Content

To determine the total flavonoid content (29), the aluminium chloride colorimetric assay was carried out, and the chemicals used were 10% aluminium chloride, 5% sodium nitrite, 10 mg of Quercetin and 1 M sodium hydroxide. One millilitre of test sample and 1 mL of standard Quercetin solution were aliquoted into test tubes, and 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution were added into each tube. After 5 minutes, 0.3 mL of 10% aluminium chloride was added. At the sixth minute, 2 mL of 1 M sodium hydroxide was added, and the volume was made up to 10 mL with distilled water and mixed well. An orange colour appeared, and the absorbance at 630 nm was measured for each sample. The concentration was determined by using the standard curve against Quercetin.

Total Phenolic Content

To determine the total phenolic content (30), the chemicals used were Folin-Ciocalteu's (FC) reagent, 8% saturated sodium carbonate solution, and 25 mg/50 mL gallic acid. The total phenolics of the extract were determined using FC reagent. Standard readings were made using a spectrophotometer at 765 nm against a black reagent. A quantity of 0.2 mL of the test sample was mixed with 0.6 mL of water and 0.2 mL of FC reagent. After 5 minutes, 1 mL of sodium carbonate was added to the solution and the volume was made up to 3 mL with distilled water. The solutions were incubated for 30 minutes at room temperature and then centrifuged. The absorbance of each sample was measured. The phenol concentration was calculated as gallic acid equivalent on the basis of a standard curve.

Total Tannin Content

For determination of the total tannin content (31), the chemicals used were FC reagent, saturated sodium carbonate solution (8% w/v), and 6.25 mg/50 mL tannic acid. The total tannin content of the extract was determined using FC method. Subsequently, 0.2 mL of the test sample was mixed with 7.5 mL of distilled water and 0.5 mL of FC reagent. A quantity of 1 mL of 35% sodium carbonate was then added, and the volume was made up to 10 mL with distilled water. The mixture was shaken and incubated at room temperature for 30 minutes. A set of reference standard solutions of tannic acid were prepared using the same method (20, 40, 60, 80, and 100 µg/mL). Absorbance was measured at 700 nm. The sample concentrations were calculated using the tannic acid standard curve.

Preparation of AS20

The AS20 formulation was prepared according to the data obtained from the qualitative and quantitative analyses of different solvent extracts of each of the parts of the plant. A combination of different extracts with high overall content of the phytocomponents was formulated. A significant challenge in creating such a combination is mixing solvents of varying polarities. Solvent extracts created by dissolving 1 g of powder in 10 mL of solvent were added in petri dishes. The mass of empty petri dish was recorded before adding the solvent. The petri dishes were air dried. After drying, the petri dish was weighed once again to determine the dry weight. Subsequently, 0.01 g/mL of dimethyl sulfoxide (DMSO) was added. After dissolution in DMSO, the desired extracts were mixed to form a polyherbal solution, AS20. DMSO was chosen due to its ability to act as a solvent for polar and non-polar compounds and its relative miscibility in a wide range of solutions.

DPPH Assay for AS20

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (32) is a colorimetric assay which is used to determine the free radical scavenging activity of a substance. The principle behind the DPPH assay is the reduction of DPPH (a free radical) which results in a purple solution being formed in the presence of an antioxidant (33). In our study, we assessed this property in the AS20 formulation. 1.0, 50, 100, 150 and 200 µL volumes of the AS20 formulation were added into five glass test tubes. Appropriate volumes of methanol were added to these to make the volume up to 1 mL. Subsequently, 3 mL of DPPH solution was added quickly into the five test tubes, which were immediately incubated in the dark at room temperature for 15 minutes. The absorbance values were measured using the spectrophotometer at a wavelength of 517 nm, and a graph was plotted against the standard. The percentage inhibition (inhibition of reactive oxygen species, such as the superoxide radical) was calculated according to the formula:

$$\%\ \text{inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

A graph of concentration (µg/mL) on the x-axis against % inhibition along the y-axis was plotted for the AS20 DPPH assay. The IC50 value was found after fitting the data to a linear equation (y=mx+c). The point at which 50% inhibition of the reactive oxygen species had occurred was hence determined (see results):

$$(50) = mx + c \rightarrow \therefore x = \frac{50 - c}{m}$$

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REFERENCES

1. Complementary Therapies. *Cancer Council*, 2020, www.cancer.org.au/cancer-information/treatment/complementary-therapies.
2. "Antioxidants and Cancer Prevention." Federal Occuptional Health, foh.psc.gov/NYCU/antioxidents.asp.
3. "*Amaranthus Spinosus*." Plants Profile for *Amaranthus Spinosus* (Spiny Amaranth), plants.usda.gov/core/profile?symbol=AMSP.
4. A Practical Field Guide to Weeds of Rice in Asia, by B. P. Caton et al., *International Rice Research Institute*, 2004, pp. 20–21.
5. Pal, Vipin Chandra, et al. "Pharmacognostical Studies of *Amaranthus Spinosus* Linn." *UK Journal of Pharmaceutical Biosciences*, vol. 1, no. 1, 2013, p. 32., doi:10.20510/ukjpb/1/i1/91112.
6. Tewari, Devesh, et al. "Ethnopharmacological Approaches for Therapy of Jaundice: Part I." *Frontiers in Pharmacology,* vol. 8, 2017, doi:10.3389/fphar.2017.00518.
7. Olajide, Olumayokun A., et al. "Anti-Inflammatory Properties Of *Amaranthus Spinosus* Leaf Extract." *Pharmaceutical Biology*, vol. 42, no. 7, 2004, pp. 521–525., doi:10.3109/13880200490893285.
8. Ahamath, J.M., et al. "Phytochemical studies and antibacterial activities of *Amaranthus spinosus* L." World Journal of. Pharmacy and Pharmaceutical Sciences, vol. 3, no. 12, 2014, pp. 1692-1697.
9. Hussain, Zeashan, et al. "Antidiarrheal and Antiulcer Activity Of *Amaranthus Spinosus* in Experimental Animals." *Pharmaceutical Biology*, vol. 47, no. 10, 2009, pp. 932–939., doi:10.1080/13880200902950769.
10. S, Harsha Vardhana. "*In Vitro* Antibacterial activity of *Amaranthus spinosus* root extracts," *Pharmacophore*, vol. 2 , no. 5, 2011, pp. 266-270.
11. ZC, Maiyo. "Antimicrobial activity of *Amaranthus spinosus* Linn." *African Journal of Biotechnology,* vol 9 (21), 2010, pp. 3178- 3182.
12. Hilou, A., et al. "In Vivo Antimalarial Activities of Extracts from *Amaranthus Spinosus* L. and Boerhaavia Erecta L. in Mice." *Journal of Ethnopharmacology,* vol. 103, no. 2, 2006, pp. 236–240., doi:10.1016/j.jep.2005.08.006.
13. Menon, Venugopal P., and Adluri Ram Sudheer. "Antioxidant And Anti-Inflammatory Properties Of Curcumin." *Advances in experimental medicine and biology,* The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease, pp. 105–125., doi:10.1007/978-0-387-46401-5_3.
14. Wink, Michael. "Modes of Action of Herbal Medicines and Plant Secondary Metabolites." *Medicines*, vol. 2, no. 3, 2015, pp. 251–286., doi:10.3390/medicines2030251.
15. Kittakoop, Prasat, et al. "Alkaloids as Important Scaffolds in Therapeutic Drugs for the Treatments of Cancer, Tuberculosis, and Smoking Cessation." *Current Topics in Medicinal Chemistry*, vol. 14, no. 2, 2013, pp. 239–252., doi:10.2174/1568026613666131216105049.
16. Tiong, Soon, et al. "Antidiabetic and Antioxidant Properties of Alkaloids from Catharanthus Roseus (L.) G. Don." *Molecules,* vol. 18, no. 8, 2013, pp. 9770–9784., doi:10.3390/molecules18089770.
17. Gülçin, Ilhami, et al. "Antioxidant Activity of Saponins Isolated from Ivy: α-Hederin, Hederasaponin-C, Hederacolchiside-E and Hederacolchiside-F." *Planta Medica*, vol. 70, no. 6, 2004, pp. 561–563., doi:10.1055/s-2004-827158.
18. Scalbert, Augustin, et al. "Polyphenols: Antioxidants and Beyond." *The American Journal of Clinical Nutrition*, vol. 81, no. 1, 2005, doi:10.1093/ajcn/81.1.215s.
19. Wink, Michael. "Modes of Action of Herbal Medicines and Plant Secondary Metabolites." *Medicines*, vol. 2, no. 3, 2015, pp. 251–286., doi:10.3390/medicines2030251.
20. Pietta, Pier-Giorgio. "Flavonoids as Antioxidants." *Journal of Natural Products*, vol. 63, no. 7, 2000, pp. 1035–1042., doi:10.1021/np9904509.
21. Amarowicz, Ryszard. "Tannins: the New Natural Antioxidants?" *European Journal of Lipid Science and Technology,* vol. 109, no. 6, 2007, pp. 549–551., doi:10.1002/ejlt.200700145.
22. Baral, Manik. "*In vitro* antioxidant activity of the whole plant of *Amaranthus spinosus*. Linn." *International journal of Biomedical and Pharmaceutical Sciences*, vol. 5, 2010, pp. 75-78.
23. Kumar, Bagepalli Srinivas Ashok, et al. "Antioxidant and Antipyretic Properties of Methanolic Extract of *Amaranthus Spinosus* Leaves." Asian Pacific Journal of Tropical Medicine, vol. 3, no. 9, 2010, pp. 702–706., doi:10.1016/s1995-7645(10)60169-1.
24. Practical Pharmacognosy, by C. K. Kokate, Vallabh Prakashan, 1994, pp. 107–113.
25. Gul, Rahman, et al. "Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra Intermedia Indigenous to Balochistan." *The Scientific World Journal*, vol. 2017, 2017, pp. 1–7., doi:10.1155/2017/5873648.
26. Ellul Micallef, Roger, and Rita Darmanin Ellul. "Determination of the Phytochemical, Antioxidant and Anti-Inflammatory Properties of Selected Maltese Medicinal Plants." *L-Università Ta' Malta*, University of Malta, 1 Jan. 2013, www.um.edu.mt/library/oar/handle/123456789/31934.
27. Le, Anh V., et al. "Improving the Vanillin-Sulphuric Acid Method for Quantifying Total Saponins." *Technologies*, vol. 6, no. 3, 2018, p. 84., doi:10.3390/technologies6030084.
28. Chang, C. "Estimation of total flavonoid content in propolis by two complementary colorimetric methods." J. *Food Drug Anal.*, vol. 10, 2002, pp. 178-182.
29. Mcdonald, Suzy, et al. "Phenolic Content and Antioxidant

Activity of Olive Extracts." *Food Chemistry,* vol. 73, no. 1, 2001, pp. 73–84., doi:10.1016/s0308-8146(00)00288-0.
30. CI, Kavitha Chandran, et al. "Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of Strobilanthes Kunthiana (Neelakurinji)." J*ournal of Medicinal Plants Studies*, vol. 4, no. 4, 2016, pp. 282-286.
31. Liyana-Pathirana, C.m., et al. "Antioxidant Activity of Cherry Laurel Fruit (Laurocerasus Officinalis Roem.) and Its Concentrated Juice." *Food Chemistry,* vol. 99, no. 1, 2006, pp. 121–128., doi:10.1016/j.foodchem.2005.06.046.
32. Huang, Dejian, et al. "The Chemistry behind Antioxidant Capacity Assays." *Journal of Agricultural and Food Chemistry*, vol. 53, no. 6, 2005, pp. 1841–1856. doi:10.1021/jf030723c.