Research Article

Proximate composition, phytochemical analysis and antioxidant capacity of *Aloe vera*, *Cannabis sativa* and *Mentha longifolia*

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Abstract
Plants, miracle of nature, are able to synthesize hundreds of chemical compounds for various metabolic functions. Numerous phytochemicals (secondary metabolites) with potential biological activity have been identified in most of the plant species. In order to determine the proximate composition, phytochemical analysis and antioxidant capacity of three well known selected plants species, a study was carried out during June 2016 in Khyber Pakhtunkhwa Peshawar. The plants under study were *Aloe vera* Linn (leaves), *Cannabis sativa* Linn (whole plant) and *Mentha longifolia* Linn (whole plant*)* in PCSIR Labs Complex Peshawar. The results from proximate analysis indicated that the plants contained crude protein in the range 0.447 to 0.953%, crude fiber ranged from 12.33 to 28.47 % and crude fat in the range of 5.87 to 14.86%. Furthermore, analysis showed the presences of important phytochemicals such as tannins, flavonoids, alkaloids, glycosides and saponins in the investigated species. Antioxidant activity of the selected plants by DPPH (2,2-diphenyl-1-picrylhydrazyl radical) scavenging assay and using ascorbic acid as a standard indicated that *Cannabis sativa* and *Aloe vera* has the strongest and nearly the same activity with IC_{50} 353µg/ml. These plants can be used as herbal products.
Keywords: Antioxidants; Chemical analysis; Phytochemicals

Introduction
Plants are miracle of nature and owned the most valuable medicinal properties. Medicinal plants are considered to be the backbone of traditional medicine. About 80% of the world's community lives in less developed countries and rely more on plants for their medical purposes. According to WHO, about 80% people in these countries regularly use these traditional medicines for their primary health requirements [1]. About 6,000 species of higher plants have been

reported in Pakistan. Out of which 12% species are known for its medicinal value [2]. Literature revealed that these plants contain active chemical constituents like phytochemicals, minerals and vitamins [3]. Phytochemical are chemicals produced by plants through primary or secondary metabolism. Medicinal plants having these bioactive chemicals with high proportion of antioxidants are considered fundamental in the prevention of a variety of degenerative diseases and have potential benefits to the people [4]. Antioxidants are chemicals that prevent oxidation and thereby remove potentially damaging oxidizing agents from a living cell. Free radicals like reactive oxygen and reactive nitrogen species (RONS) are molecules or molecular fragments having an unpaired electron. RONS is a combined term and consists of two classes, reactive oxygen species (ROS) and reactive nitrogen species (RNS). These free radicals are considerably unstable and extremely reactive due to the presence of unpaired electrons. During the sequence of chemical reactions that produce energy for our cells in mitochondrial respiration, ROS and RNS are naturally produced as byproducts of this essential process [5]. Antioxidants protect body against the harmful effects of uncontrolled reactive oxygen species and counteract their side effects. A number of radical scavenging antioxidants are common in food sources like fruits, vegetables and tea, etc [6].

Aloe vera Linn (*A. vera*). contains more than 75 nutrients and 200 active compounds, including minerals, enzymes, vitamins, sugars, amino acids, saponins, anthraquinone lignin and salicylic acid [7]. In conventional medicine, its gel has been applied in the cure of skin complaints like wounds, burns, skin irritations and pimples. It has also been used in constipation, headache, ulcers, arthritis, asthma, jaundice, diabetes and coughs. The bioactive compounds present in *A. vera*

possess analgesic, antiseptic, antibacterial, anti-diabetic, antioxidant, anti-inflammatory and anticancer properties [8, 9]. Aloin, mixture of glycosides, is the active ingredient of various drugs. Despite this, the cosmetic and medicinal industries regularly use *A. vera* for soothing, moisturizing and healing ability [10]. It has also been used in the manufacture of lotion, soaps, shampoos, powders, creams, facial cleansers, capsules and so many other products both for external and internal uses [11]. Plant helps in nervous system functioning, enzymes activation, regulation of body liquids and so on due to the presence of calcium, magnesium, zinc, manganese, chromium, sodium, potassium etc [12, 13].

Cannabis sativa Linn (*C. sativa*) contains about 104 cannabinoids which are the most attractive constituents [14]. Its seed contains carbohydrates about 30%, protein 25%, insoluble fiber 15%, potassium, phosphorus, sulphur, calcium, magnesium, iron, zinc along with vitamin B6, B1, B2, B3, D, E and C [15]. Extracts and tinctures obtained from *C. sativa* were used for numerous different complaints including pain, whooping cough, asthma, and as a sedative/hypnotic agent [16]. Extracts are also used to treat glaucoma, AIDS, eye problems, muscle spasticity, insomnia, convulsion, depression, hypertension and treatment of pain [17, 18]. These cannabinoids are also proved to delay and prevent the growth of cancer cells [19].

Mentha longifolia Linn (*M. longifolia*) is used for the treatment of bronchitis, flatulence, liver complaints and ulcerative colitis due to its anti-inflammatory, stimulant, emmenagogue, carminative, antioxidant, antispasmodic, cytotoxic and anticatharral activities [20]. Its extracts are used for relieving cold, cough, headache, stomach cramps and indigestion [21]. It is good for the treatment of whooping cough, muscle spasm, asthma, skin ulcer and hemorrhoids. It is also used for the treatment

of rheumatism and other painful infections [22].

Materials and methods

Collection and identification of plant materials

Fresh plant samples of *A. vera* Linn, *C. sativa* Linn and *M. longifolia* Linn were collected from the botanical garden of Pakistan council of scientific and industrial research laboratories complex (PCSIR) Peshawar and *Palosi* fields Peshawar. The plants were identified by Dr. Arshad Hussain (Senior Scientific Officer) PCSIR Labs Complex Peshawar. All the chemicals and equipments were provided by PCSIR Labs Complex Peshawar. The collected leaves (*A. vera*) and whole plant (*C. sativa* and *M. longifolia*) were first cleaned with running tap water and then with distilled water to remove dust and sand etc. These plants materials were air dried in shade for few days. The dried plant materials were crushed to fine powder in an electric blender, stored in polythene bags and were tagged. Then different extracts were made ready by using the standard methods [23].

Preparation of extract for proximate and phytochemical analysis

Plant material (5g) was dissolved in about 90 ml water and boiled for15 minutes, cooled and was filtered by using Whatmann filter paper No. 42 and then 100 ml volume was made in volumetric flask. This solution was used in phytochemical and proximate analysis procedures.

Preparation of extracts for antioxidant analysis

Plant sample of 5g was dissolved in 100 ml of acetone and kept on orbital shaker for one week. The extract were then concentrated on rotary evaporator at 40°C and stored for antioxidant assay determination.

Proximate analysis

Proximate analysis of the selected medicinal plants involved determination of moisture, ash, crude protein, crude fat, crude fiber, pH, total acidity and total soluble solids. All these were found out by manual methods [23].

Phytochemical screening

Phytochemical screening for the presence of tannins, alkaloids, flavonoids, triterpenoids, saponins, glycosides and steroids was carried out qualitatively [24, 25].

Alkaloids

The presence of alkaloids was confirmed by the following tests.

Mayer's test

To aqueous extract in a test tube, small amount of Mayer's reagent was carefully added along the walls. Development of creamy precipitate indicated alkaloids in the sample.

Wagner's test

To each aqueous extract in test tube, about 1 ml of Wagner's reagent1 was added drop wise. Formation of reddish brown ppt was an indication for the presence of alkaloids.

Flavonoids

The presence of flavonoids was confirmed by the following tests

Ammonia solution test

To the aqueous extract, little amount of 1% ammonia solution was added to the test tube. Existence of flavonoids was detected by the formation of yellow colour.

Ferric chloride test

To the extract of plant material in a test tube, a number of drops of $FeCl_3$ solution were added, formation of green/black coloration was an indication for flavonoids.

Tannins

In a test tube, about 0.5g crushed plant material was heated to boiling with 20 ml of distilled water, followed by the addition of 0.1% $FeCl_3$, formation of blue or black color indicated tannins in the sample.

Saponins

Powdered sample of 2g was boiled with 20 ml of distilled water for some time. Extract of 5 ml was shaken vigorously with 10 ml distilled water for 10 minutes. Persistence of

froth on heating was an indication for the presence of saponins.

Triterpenoids

In 2 ml chloroform ($CHCl_3$), about 5mg of the powdered sample was added, followed by the addition of 1 ml acetic anhydride very carefully along the walls and 1 ml sulphuric acid (H_2SO_4). Formation of reddish violet color showed the presence of triterpenoid.

Steroids

Powdered sample (1gm) was added in10 ml chloroform and concentrated H_2SO_4 (1 ml) was added into the tube using side walls. Two layers were formed, upper layer became red and the lower layer of H_2SO_4 displayed yellow color with green fluorescence and indicated steroids.

Glycosides

Extract of plant samples was reacted with hydrochloric acid followed by neutralization with sodium hydroxide. Then some amount of Fehling A and Fehling B was added. Formation of red precipitates indicated glycosides.

Antioxidant assay (DPPH scavenging activity)

Stable DPPH (2,2-diphenyl-1-picrylhydrazyl radical) radical was used as a reagent in this method which shows absorbance at 517 nm on UV visible spectrophotometer and the presence of antioxidant in test solution decreased its concentration with time. This results in the disappearance of absorption and colour change from purple to yellow. In 95%methanol, 0.004% w/v DPPH solution was made for present research work. Ascorbic acid (vitamin C) was used as reference standard [27].

DPPH scavenging (%) was calculated by following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of the control} - \text{sample absorbance}}{\text{absorbance of the control}} X 100$$

Results and discussion

The results (Table 1) of proximate analysis of *A. vera*, *M. longifolia* and *C. sativa* revealed that moisture content was found highest (6.8%) in *A. vera*, 5.5% *C. sativa* and 5.2% in *M. longifolia*. Water is the major part of the body cells. It helps to cushion and lubricate the brain and the joints and also helps to transport nutrients and waste. Water helps in regulation of body temperature and blood pressure.

Ash content was analyzed in the range of 3.17 to 4.33%. Maximum ash was found in *C. sativa* and minimum in *M. longifolia*. Ash is actually the residue left after all the moisture and organic matter has been removed at high temperature. High ash content of these plants is actually a measure of the mineral richness [26]. Maximum crude fat was studied in *A. vera* and minimum in *M. longifolia*. In living organisms, fat are the usual stored forms of energy. They are the main structural element of phospholipids and sterol [28].

Crude fiber contents showed the maximum value for *M. longofolia* 28.47% and minimum 12.33% for *A. vera*. Crude fiber enhances digestibility and is considered excellent in the cure of gastrointestinal disorders, diabetes, obesity and cancer [29]. Similarly crude protein percentage of medicinal plants (Table 1) were found highest (0.95%) for *A. vera*, followed by *C. sativa* (0.76%) and lowest (0.45%) in *M. longifolia*. Total sugar contents and total acidity (Table 1) in selected plants were in the range of 07.60% to 1.90% and 03.04 to 02.02 respectively. Total soluble solids were analyzed in the range of 1.57 to 0.01. A slight acidic pH of 10% solution of all plants were obsereved in the range of 4.83 and 3.90.

A. vera was investigated for different parameters including proximate analysis and reported 7.3% moisture, 8.72% ash, 5.4%

crude fat 8.4% crude fiber and 16.2% crude protein. The reported data was in accordance with the present research findings [30]. The proximate analysis of the *C. sativa* plant parts separately and reported 6.87 % moisture, 11.18% ash, 23.78% crude proteins and 18.95% crude fiber in the leaf. Likewise in stem, 17.20 % crude protein, 23.13% crude fiber, 6.78% ash and 5.16% moisture was determined. While in seeds of *C. sativa* 20.19% crude protein, 25.36% crude fiber, 5.91% moisture and 7.20% ash was measured [31].

The proximate analysis of four medicinal plants [32] including *M. longifolia* and reported the highest crude protein value of 7.49% and 2.34% of crude fat in *M. longifolia* [22] which were in complete accordance with present data while moisture content was lower than present results. The difference in moisture content may be due to climatic condition. As water content of plants depends on the nature of the soil, the climatic condition and part of the plant used [33].

Table 1. Proximate analysis of *Aloe vera***,** *Cannabis sativa* **and** *Mentha longifolia*

S. No.	Constituents	*A. vera* (leaves)	*C. sativa* (whole plant)	*M. longifolia* (whole plant)
1	Moisture (%)	6.8±0.43*	5.5±0.49	5.2±0.82
2	Ash (%)	03.66±0.50	04.33±0.58	03.17±0.29
3	Crude fat (%)	14.86±1.02	11.17±1.14	05.87±0.35
4	Crude fiber (%)	12.33±1.15	16.87±0.35	28.47±0.81
5	Crude Protein (%)	0.95±0.31	0.76±0.35	0.44±0.38
6	Total sugar	01.90±0.20	03.40±0.17	07.60±0.35
7	Total acidity(10%sol)	2.02±0.002	2.3±0.002	03.04±0.01
8	Total S S(10% sol)	0.01±0.004	01.57±0.31	0.40±0.03
9	PH(10% sol)	04.7±0.05	04.83±0.35	03.90±0.26

*Mean± S.D (n=3)

Phytochemical analysis

Phytochemical analysis of the selected plant samples were carried out using suitable procedure [24, 25]. Plants were examined (Table 2) for tannins, saponins, alkaloids, flavonoids, triterpenoid, steroids and glycosides.

Alkaloids, flavonoids, saponins, steroids, triterpenoids were detected in all the three plants while tannins were absent in *M. longifolia.* Glycosides were detected only in *C. sativa* and absent in rest of the plants. Thus maximum number of phytochemicals (secondary metabolites) was present in *C. sativa*. Different phytochemicals have been revealed to perform a large variety of actions. Like alkaloids protect against chronic diseases [34]. Alkaloids are beneficial to plants and serve as repellant to parasites and predators and show antimicrobial and pain

relieving property [35]. Tannins, flavonoids, phenol, alkaloids and saponins have also been determined quantitatively in appreciable amounts in the leaf of *A. vera* [36], while some confirmed the absence of glycosides in *A. vera* leaf [37]. The content of steroids, alkaloids, cardiac glycosides, flavonoids, terpenoids and steroids in the leaf extract of *C. sativa* while saponins and tannins were absent in the said extract [38]. The presence of alkaloids in these plants like *C. sativa* verifies their traditional use as insect repellant and confirms their antimicrobial activities.

Flavonoids and tannins are phenolic compounds and plant phenolics are the compounds that perform significant antioxidants activity and have anti-inflammatory properties [39]. Tannins exhibited antitumor, antibacterial,

antidiabetic and anti-inflammatory activities. Certain tannins are also stated to hinder HIV replication [40]. Saponins are glycosides and present widely in plants. Saponins have antibiotic properties and protect against hypercholesterolemia and hyperglycemia. They possess anti-inflammatory, antioxidant, anticancer and central nervous system activities [40]. Steroids and triterpenoids show analgesic properties. Plant steroids are significant for their cardio tonic and central nervous system activities, analgesic, anti-inflammatory, insecticidal and antimicrobial properties [41]. These plants are used traditionally for the treatment of cough, headache, inflammation, wound healing, expectorant, gastrointestinal disorders, carminative, asthma, antiseptic, antidiabetic, weight loss and so on due to the presence of secondary metabolites.

Table 2. Phytochemical screening of *Aloe vera*, *Cannabis sativa* and *Mentha longifolia*

S. No.	Parameter	*A. vera* (leaves)	*C. sativa* (whole plant)	*M. longifolia* (whole plant)
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Tannins	+	+	−
4	Saponins	+	+	+
5	Triterpenoid	+	+	+
6	Steroids	+	+	+
7	Glycosides	−	+	−

+: Present- : Absent

Antioxidant activity

Antioxidant activity of the acetonic extract of the selected plants was measured at various concentrations from 100 to 500µg/ml. The scavenging of free radicals (%) of plant extracted samples (Table 3) of *A. vera, C. sativa* and *M. longifolia* showed significant antioxidant property of 59% for *M. longifolia*, 61% for *C. sativa* and 64% for *A. vera* at 500 µg/ml. Ascorbic acid was used in the research work as a standard antioxidant for all the selected plant extracts and displayed (Table 3) 38, 53, 68, 83 and 84 (%) antioxidant activity at 100-500 ug/ml concentration respectively. The data revealed that *A. vera* and *C. sativa* displayed strongest antioxidant activity in plants. Different methods are available for assessing the antioxidant activity of plants. The most simple, expedient and convenient assay for the determination of antioxidant property of medicinal plants is the radical scavenging method, with stable DPPH (1, 1 diphenyl-2-picrylhydrazyl) radical by spectrophotometer. DPPH radical get an electron from antioxidant and decreases the absorbance [42]. For screening antioxidant activity, DPPH is extensively used because of its accommodation many samples and its sensitivity for detection active ingredients even in very low concentration [43]. Ascorbic acid has enhanced scavenging ability of DPPH than extracts. It was recognized that antioxidant property of the acetonic extracts (Table 3) of selected medicinal plants depends on concentration. Data revealed that radical scavenging activity (Figure 1) increased with increase in concentration.

Inhibition concentration (IC_{50}) parameter (Table 4) was employed in elucidating results from DPPH process. IC_{50} is actually the extract concentration at which 50% inhibition of DPPH achieved. It is negatively linked with the scavenging ability and expressed the antioxidant amount necessary for decreasing 50% radical. The lesser the IC_{50} value of the extract, the greater will be its antioxidant

activity. The IC$_{50}$ (µg/ml) was found 428 for *M. longifolia*, 353 for *A. vera* and *C. sativa*. IC$_{50}$ was obtained from linear regression curve (Figure 1) of concentration against the percent scavenging activity. Literature exposed that the antioxidant property of plants is due to the presence of phenolic compounds such as terpenes, flavonoids, saponins and poly phenol which has free radical scavenging activity. The presence of tannins and flavonoids in majority of plants was responsible for the observed free radical scavenging effects [44].

Table 3. DPPH radical scavenging activity (%) of acetonic extracts of *Aloe vera*, *Cannabis sativa* and *Mentha longifolia*

Conc (µg/ml)	*A. vera*	*C. sativa*	*M. longifolia*	Vitamin C
100	24±0.70*	26±0.55	16±1.12	38±1.01
200	38±1.64	37±1.00	24±0.55	53±.1.08
300	47±0.64	46±0.90	36±0.45	68±1.18
400	53±1.52	55±1.12	44±0.79	83±1.43
500	64±1.21	61±0.85	59±1.02	84±1.62

*Mean±S.D (n=3)

Table 4. DPPH radical scavenging activity of *Aloe vera*, *Cannabis sativa* and *Mentha longifolia* (IC 50 in µg/ml)

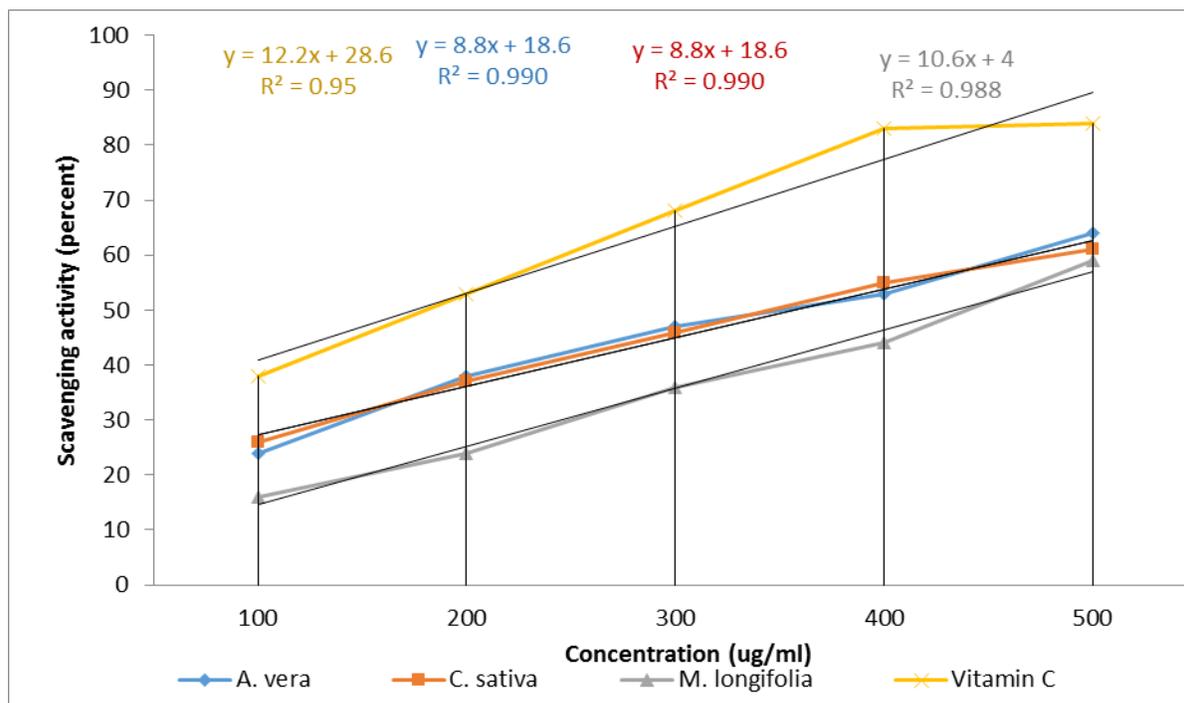| S. No	Acetonic extracts	IC 50
1	*A. vera*	353µg/ml
2	*C. sativa*	353 µg/ml
3	*M. longifolia*	428 µg/ml
4	Vitamin C	167 µg/ml

Figure 1. Antioxidant activities (DPPH) of *A. vera*, *C. sativa* and *M. longifolia*

Conclusion

The present research work is an effort in confirming the nutritive value, phytochemical combination and antioxidants ability of selected plants. Proximate analysis revealed that these plants are good source of nutrients like proteins, fiber and fat. Most of the plants contained phytochemicals like alkaloids, tannins, flavonoids, glycoside etc which have good pharmacological effect. Among the selected medicinal plants, *C. sativa* has shown the presence of all tested phytochemicals. Both *C. sativa* and *A. vera* has shown significant antioxidant activity. Thus these plants could be utilized in food and as herbal products.

Author's contributions

Conceived and designed the experiments: Z Waris & Y Iqbal, Performed the experiments: Z Waris, Analyzed the data: A Hussain, Shafqatullah & AA Khan, Contributed reagents/ materials/ analysis tools: MW Khan & A Ali, Wrote the paper: A Ali.

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