ASSESSMENT OF ANTIOXIDANTS AND ANTIMICROBIAL ACTIVITIES OF FRUIT EXTRACTS OF *DUCHESNEA INDICA*

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ABSTRACT

Duchesnea indica, a rosaceae plant, is a perennial herb widely distributed different parts of world. Ethnobotanical study is an important activity related to the research and development of drugs. The growing need to find alternatives for the treatment of chronic degenerative diseases, such as diabetes, hypertension, and metabolic syndrome, among others, justifies the study of medicinal plants used in traditional medicine A study was conducted by using different fruit extracts of *Duchesnea indica* for different assays . Chemical analysis of the fruits revealed significant protein and oil contents fiber content The significant quantity of flavonoids, alkaloids, tannins, saponins and phenols also found in extracts . The scavenging ability of the fruit extracts was evaluated using various solvent based assays, it was found that fruit exhibited highest antioxidant activity. The antimicrobial activity of the leaf extracts were tested against both and positive microorganism . The methanolic extract exhibited higher inhibitory activity against gram negative bacteria. It was observed that *Duchesnea indica* fruit extracts may serve as a valuable source of natural antioxidants and hold potential for to supply pharmaceutical raw material to industry for antimicrobial/ anticancer drugs formulation etc.,

Key words: *Duchesnea indica* , secondary metabolites, anti-oxidant, anti-microbial potential.

INTRODUCTION

Pakistan occupies unique position in map. It has diverse climatic conditions having considerable potential within the variety of medicinal plants (Khan and Gul, 2007). Considerably, plants produce chemicals, for example, essential and optional metabolites, which have huge applications in current treatment. Indigenous individuals generally depend on conventional drugs got from therapeutic plants. These plants have the ability to assimilate an assortment of dangerous components. The ingestion of such plants for therapeutic reason can have basic reactions. Subsequently, as to the toxicological thought of therapeutic plants, an exertion has been made to survey the essential substance of ethno therapeutically vital plants of Pakistan and to highlight the current holes in learning of the wellbeing and viability of customary natural drugs.

Plants vary in their chemical and phytonutritional composition because of their genotype, the reason why one chemical compound active in one plant is not present in other plant. Chemical composition of plants also varies because of the soil in which they are growing and the type of fertilizer which is provided to them.

Strawberries are a rich wellspring of polyphenols which add to berry shading and plant illness resistance, and have been appeared to bring down the risk of many diseases when consumed. While a significant assortment of work exists on the polyphenolic organization of commercial strawberry (*Fragaria x ananassa*), less data is accessible concerning polyphenols in wild strawberry (*Duchesnea indica*). (Archbold,2016)

Duchesnea indica belongs to family Rosacea. It is somewhat similar to true strawberry *Fragaria ananassa,* but apparently it seems the independent evolution of similar fruit type. Native home of *Duchesnea indica* is Eastern and Southern Asia but it also used as an ornamental plants in other parts of world.The plant is used as anticoagulant, antiseptic and to reduce fever in traditional medicines. The leaves are used to cure swellings while flowers are helpful for blood circulation and fruit for skin related disease. The mixture of the plant is also used for abscesses, boils, burns etc. (Duke et al., 1985). In *D. indica*, which include ellagitannins, ellagic acid and ellagic acid glycosides, hydroxybenzoic acid and hydroxycinnamic acid derivatives, and flavonols are being identified(Zhu et al., 2015). In recent times the interest in medicine obtained from natural source is increased widely because of their high efficiency and less side effects. Antioxidants are the molecules that inhibit the oxidation of other molecules. Oxidation is a process in which electron is lost and results in free radicle formation. The formed free radicle thus initiates a chain reaction by forming more free radicles. Free radicals can cause different kind of diseases including cancer, heart disease, brain disorders, Alzheimer's disease, cognitive impairment (Kinnula and Crapo, 2004) (Singh and Jialal, 2006) (Sas et al., 2007) (Smith et al., 2000) (Guidi et al., 2006). Human body is based on two major type of systems i.e. enzymatic and non-enzymatic antioxidant system, which defend body against free radicle formation. If body is impaired to form antioxidants then they are also taken through antioxidant supplements, as well as various food sources are enrich in antioxidants. Ethno therapeutic conventional learning about the employments of indigenous medicinal plants for treating different human infections is absolutely in hold of the elder community member. The new generation is very little mindful about these critical conventional therapeutic practices (Ejaz et al., 2015) Many microbial diseases are cured by antibiotics. But prolong use of antibiotics leads to drug resistance and thus the efficacy of drug is reduced. .

Drugs from these plants are inexpensive, safe, efficient, and rarely accompanied by side effects. Plant derived antimicrobials are, among others, in the focus of research because of their easy accessibility and wide antimicrobial spectrum. Usually several ingredients with different target sites are responsible for the antimicrobial properties which decreases the possibility of development of microbial resistance (Al-Zahrani et al., 2016).The spread of multiple drug resistant pathogens has led scientist to develop new antibiotics. As with time bacterial and fungal infection becoming more and more common. *Duchesnea indica* naturally produce antimicrobial compound as defensive substance against plant pathogen.sa These compounds prevent plants naturally from development of bacteria and fungus from developing (Rosa *et al*., 2003). Therefore this study was conducted with aims and objectives. (1) Compositional analysis of fruit of *Duchesnea indica* (2). Assessment of fruit extracts for various bioactive compounds (3). Bioactivity assessment of fruit of *Duchesenea indica.*

MATERIALS AND METHODS

Collection of Samples

The leaves and fruit samples of *Duchesne indica* will be collected from hilly areas surrounding Islamabad during 2023. The leaves and fruit samples will be collected in fine plastic bags dully labeled with name, date and areas of collection of samples. The samples will be properly identified by expert taxonomist and will register as specimen for future reference.

Preparation of plant Samples

The leaves and fruits samples will be shadow dry and sun drying following by oven dried for over night at 60 C. The dried samples will be ground by using electrical grinder and sieve (80 msh) and save in plastic bags for further uses.

Preparation of plant Extracts

Methanolic and ethaolic, extracts was prepared by using 50 grams each of plant samples of plant extracts by using Soxhlet apparatus and rotary evaporator techniques followed by shaking for over night followed by filtration .

PROXIMATE ANALYSIS

For proximate analysis the parameters determined were moisture, crude fat ,crude protein,crude fibre and carbohydrates. All these analysis done by the method explained by (Rao et al., 2007)

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

Preparation of plant Extracts

Five grams of dried at long last powdered plant taken in a container and include 200ml of refined water. Heat on a hot plate with consistent mixing at around 30º - 40º C for 20 mins.Water extricate transformed into sifted utilizing channel paper and the filtrate got to be utilized for the phytochemical examination. The water extricate got to be spared in fridge for further assays (Sofowra,1993; Trease et al.,1989)

Test for Proteins

Ninhydrin test: Crude extracts boiled with 2 ml of O.2% of Ninhydrin solution and violet coloration indicated the presence of amino acids.

Test for Reducing Sugars

Fehling's test: Solution of Fehling A and Fehling B reagents mixed and 2ml of this mixture was added to crude extract followed by boiling. The brick red precipitate indicate the presence of reducing sugars.

Test for Carbohydrates

a) **Benedict's test:** Benedict's reagent 2ml included into concentrate and heated till it start boiling. Appearance of Reddish brown precipitate shows the presence of carbohydrates.

Test for Phenol and Tanins

Crude extract became mixed with 2% solution of NaOH. An excessive yellow colour became fashioned which became colorless on addition of few drops of diluted acid, indicated the presence of phenols and tannins.

Test for Flavonoids

2ml of 2% solution of NaOH added into Crude extract. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid, indicated the presence of flavonoids.

Test for Saponins

Crude extract and 5ml of distilled water added in a test tube and shaken vigorously. The foam formation is the indication of the presence of saponins.

Test for Glycosides

Liebermann's test

Crude extract had been combined with each of 2ml of chloroform and 2ml of acetic acid. The mixture became cooled in ice and changed into cautiously added with focused H2SO4. A colour trade (violet blue to green) indicated the presence of steroidal nucleus, i.e. glycine portion of glycosides.

Test for Steroid

Crude extracts were blended with 2ml of chloroform and H2SO4 become introduced on sides of glass tube. A red color produced in the decrease chloroform layer indicated the presence of steroids.

Test for Terpenoids

Crude extract and 2ml of chloroform mixed together and then evaporated to dryness.After that 2 ml of concentrated H_2SO_4 was added and heated for 2 minutes. The grayish color is the indication of terpenoids.

Test for Alkaloids

Crude extract become blended with 2ml of 1% HCl and heated lightly. Mayer's and Wagner's reagents had been then introduced to the mixture. Turbidity of the ensuing precipitate become taken as evidence for the presence of alkaloids.

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Determination of Alkaloids

Total 5g of sample was added to 10% acetic acid (20ml acetic acid + 180ml ethanol). Cover and stand for 4 hrs. Then Filter the solution and filtrate placed on water bath evaporate it one quarter. Then add concentrated ammonium hydroxide drop by drop and left the solution for settlement. Then again filter the solution. Wash precipitates using dilute ammonium hydroxide collected on filter paper. Pre weigh the filter. Dry in oven and calculate the percentage by difference in weight (Haborne, 1973).

Determination of Saponins

About 10 g of test and 100ml of 20% aq ethanol are combined. Place them in water bath and stand it for 4 hrs with continuos mixing at 55º C. Channel the arrangement. Re-Extract with 200ml of 20% ethanol. The consolidated concentrate lessened upto 40ml on water shower at 90ºC. The moved concentrate put in sepratory funnel. Include 20 ml of di-ethyl ether took after by

enthusiastic shaking. 2 layers were obtained,discard the ether layer and watery layer recuperated. Cleansing procedure did over and over. The consolidated concentrate were washed out twice with 60 ml n-butanol containg 10ml 5% sodium chloride watery. Dispose of sodium chloride layer and concentrate transfereed to pre measured recepticles and warmed in water shower to dissipate arrangement.. The sample was oven dried, weight and content of Saponins determined as percentage (Obadori and Ochuko, 2001)

Determination of Flavonoids

Total 10g of sample extracted with 80% methanol at room temperature. The filterate transferred in pre weight falcon tubes. Evaporation performed in water bath. The extracyed material calculated in percentage. (Bohn and Kocipai, 1994)

Determination of Tanins

Tanins were estimated by colorimetric test. Extraction of 0.125g of test was performed with 25ml of Acetone(70%) in Mechanical shaker for 12 hrs. In the wake of separating the arrangement 0.5ml of Folin-Ciocalteu reagent and 2.5ml of Na_2CO_3 included. Absorbance was measured by utilizing spectrophotometer at 725nm. Tanins in concentrate was measured utilizing standard bend results and tannic corrosive was utilized as standard (mg/g of removed compound). Investigation was done in triplicates (Pearson, 1976)

Determination of Total Phenolic Contents

The measure of phenols in concentrate was measured by Folin – Ciocalteu reagent. 5 grams powder test and 200 ml of refined water combined. Warming and continues mixing was performed at 30ºC-40ºC for 20 minutes on hot plate. The course of action was filtered and 1 ml of concentrate , 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% plan of Na2CO3 was incorporated. The mix was left at room temperature for 15 minutes. Test absorbance was determined using spectrophotometer at 765nm by differentiating and gallic destructive (1mg/ml) that was used as clear. Complete polyphenol content in concentrate was measured using standard as gallic acid equivalent (mg/g of isolated compound). Examination done in triplicates. (Harborne, 1973).

ANTIOXIDANT ASSAY

To calculate the antioxidant activity in fruit and leaves of *Duchesnea indica* four methods are used.

DPPH Radical Scavenging Activity Assay

To strategy clarified by Chew et al., 2008 is to decide antioxident action of test. 1mg unrefined concentrate was broken up in individual solvents. totally distinctive focuses were made of the stock. 1ml of each focus were taken in tube and 2ml of DPPH (2.4mg DPPH + 100ml Methanol) was intercalary. When thirty minutes of hatching at temperature absorbance was measured at 517nm wavelength. water-dissolvable vitamin was utilized as would be expected. DPPH is lightweight delicate in this manner the trial should be exhausted dimness. the capacity of the example to assemble the DPPH structure decide by taking after condition:

$$Scavenging\ effect(\%) = \frac{(Control - Sample)}{Control}$$

ABTS Assay

The method explained by (Re *et al*.,1999)used for ABTS assay is used. ABTS and potassium persulfate added in distilled water in a concentration of 7 mM and 2.45 mM individually. These two arrangements were mixed and the mixture permitted to remain oblivious at room temperature for 16 h before use with a specific end goal to deliver ABTS radical (ABTS•+). For the investigation of phenolic intensifies the ABTS radical arrangement was weakened with refined water to an absorbance of 1.00 at 734 nm. At that point ABTS arrangement was added to test and the absorbance perusing was taken 6 min in the wake of blending utilizing the spec¬trophotometer. Results are presented as the ability of phenols to scavenge 50% of free radical ABTS • + (IC50) .

All determinations were carried out in triplicate.

Reducing Power Assay

Reducing Power of the concentrates were dictated by utilizing the strategy portrayed by (Ullah et al.,2013).Stock arrangement were set up by dissolving 4mg in 1 ml of separate solvents. Distinctive fixation were taken from stock.. 400µl of phosphate cradle (0.2M,pH 6.6) and 500µl of potassium ferricyanide(1%) were included response blend. Hatch the response blend for 30 minutes at 50ºC and after brooding include 500µl of 10% of trichloric corrosive. Absorbance was measured at 700nm. Ascorbic corrosive is utilized as positive control.

ANTIMICROBIAL ACTIVITY

Preparation of Extract For Antimicrobial Activity

The powder sample was extracted with solvents like methanol and ethanol. Extraction of sample was carried out by 24 hrs shaking and re-extracting. Then sample left to dry. When solvent evaporated, crude extract obtained.

Microorganisms Tested

Antimicrobial activity was tested against gram neagative *Escherichia coli, Stephylo aureus, Pseudomonas aeruginosa* and *Salmonella typhi* bu agar well diffusion method. Bacterial inoculums were prepared in autoclaved Lauria-Bertini media gL^{-1} in different vials. The inoculum was incubated for 24 hours at 37°C for maximum growth.

Statistical analysis

Data obtained after analysis was for subjected to further statistically analyzed

RESULTS AND DISCUSSION

PROXIMATE ANALYSIS

Proximate analysis is used to analyze the five basic components of biological sample i.e. Moisture or water content, Crude protein , crude fat, crude fibre and total carbohydrates. These tests also give us idea about the nutritional contents of the sample in cheap way. But verification of nutition panel is necessary.

The result of phytochemical analysis is shown in respective tables and figures . Fruit contain large amount of crude protein and carbohydrates i.e. 14.2% and 18.16% but this amount is

comparatively less then leaves. Moisture content in fruit is 5.5%, crude fat 6.32% and crude fiber which indicates that fruit sample is rich in carbohydrates and low moisture content is present in samples.

Table 1. Proximate Analysis (%) of Leaves and Fruits of *Duchesnea indica*

	Fruits
Moisture	5.5± 1.3
Crude Protein	1.2 ± 0.01
Crude Fat	0.001±0.08
Crude Fibre	3.0±0.005
Carbohydrates	8.0±0.02

Result are Mean ± Standard deviation (n=3)

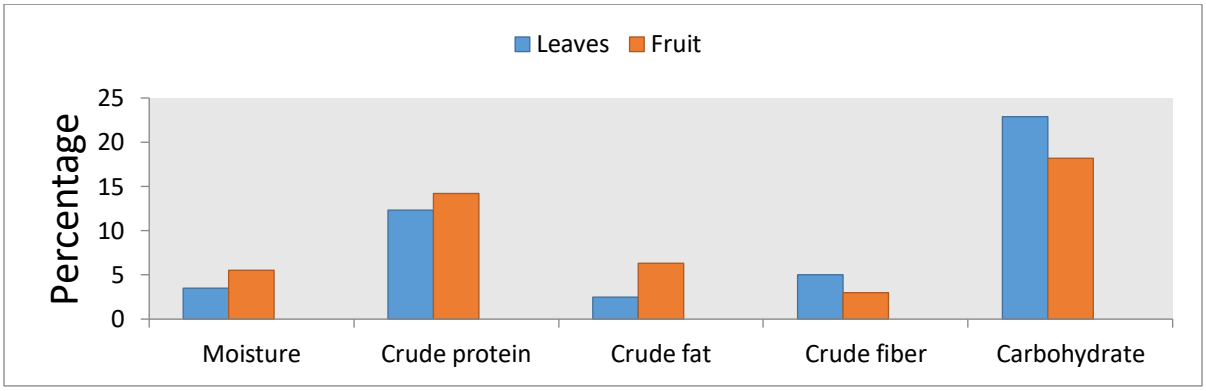

Figure 1. Proximate analysis of leaves and fruit of *Duchesnea indica*

QUALITATIVE ESTIMATION OF PHYTOCHEMICALS

Phytochemical analysis of *Duchesnea indica* revealed that both leaves and fruit are good source of major bioactive compounds which include flavonoids, tanins, saponins,alkaloids and phenols. The presence of these bioactive compounds also reveals the medicinal importance of its both fruit and leaves. The fruit is rich with phenol contents and thus can be used as immune enhancer against various diseases. The presence and absence of various bioactive compounds is shown in Table 2. comparatively high amount of tannins in comparison with fruit. The leaves contain (26.56±4.5)mg and fruit contain (1.22±0.084)mg per 100g of sample. The standard used for this analysis was Tannic acid. Caliberation curve of tannic acid is shown in following figures.

Table 2. Qualitative estimation of phytochemicals of *Duhesnea indica* **extracts fruits**

S.No	**Phytochemicals**	**Fruits**
1	Alkaloids	+
2	Carbohydrates	+
3	Saponins	+
4	Flavonoids	+
5	Protein and amino acids	+
6	Reducing sugar	+
7	Phenol and tanins	+

8	Cardiac glycosides	+
9	Steroids	+
10	Terpenoids	+
11	Glycosides	+

+ Present - Absent

QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS

Quantitative estimation of phytochemicals is important to understand the medicinal and nutritional value of particular plant. Most common phytochemicals are Flavonoids, Phenols, Saponins, Tanins and Alkaloids. Phenols exhibit multiple pharmacological functions out of which most important are their antioxidant activities. Data in Table 3, shows the amount of phytochemicals in *duchesnea indica* in mg of per 100g of sample. The amount of flavonoids in leaves is 20.56±0.67mg/100g of sample and fruit contain 42.8±1.05 mg/100g of sample. Total phenols were estimated by the method explained by (Harborne,1973) using gallic acid as standard. The standard curve of gallic acid . According to that leaves of *Duchesnea indica* contain less amount of total phenols in comparison with leaves i.e. 84.4±8.3 mg of per 100g of sample while higher amount of total phenols observed in fruit contain 517±214.3 mg of per 100g of sample. Phenols play an important role in growth of both tissue and cells of humans and animals. Phenols are also immune enhancers.

Saponins are glucosides.Mostly found in vegetables, beans and herbs. Saponins can also act as immunity booster. Studies also reveal their effect on blood cholesterol level, bone loss and antitumor activity. The amount of Saponins in *Duchesnea indica* leaves is comparatively higher then fruits i.e. leaves (20.4±4.5) mg and fruit (0.78±0.1) mg per 100 g of sample. Whereas the higher quantity of alkaloids (5.1±0.42) mg was discovered in leaves then fruit (4.8±0.2) mg per 100 g of sample. Tannins are phenolic compounds and thus they can show antioxidant activities. Due to their antioxidant nature they also possess anticancer and antimutagenic potential. When the tannin concentration was analyzed in *Duchesnea indica* it is found that leaves of *Duchesnea indica* have

Table 3. Quantitative estimation of phytochemicals (mgGAE/100g) in *Duchesnea indica* Leaves and Fruits

	Flavonoid	Phenol	Saponins	Tanins	Alkaloids
Leaves	20.56±0.67	84.4±8.3	20.4±0.53	26.56±4.5	5.1±0.42
Fruit	42.8±1.05	517±214.3	0.78±0.1	1.22±0.084	4.8±0.2

Values expressed as mean ± Standard Deviation (n=3)

ANTIOXIDANT ASSAY

DPPH radical scavenging activity assay

DPPH is the most common assay used to determine antioxidant activity of sample. It give perfect band at the absorption of 517nm. The standard used for this assay was Ascorbic acid. It was observed that fruit of *Duchesnea indica* is the better source of antioxidents then leaves as well as the methanolic extract of both leaves and fruit give better result in comparison with ethanolic extract. The result of DPPH explained in form of IC50 that is the minimum concentrations require to attain 50% activity. The IC50 value of methanolic extract of leaves was (46.2±0.3) µg/ml and of ethanolic extract of leaves was (54±0.5)mg/ml. And the IC50 value of methanolic fruit was observed as (38±0.8)µg/ml and of ethanolic fruit extract was (50.3±0.44) µg/ml. The detailed results of leaves and fruit of *Duchesnea*

indica are expressed The percent scavenging activity of both leaves and fruits in comparison with the scavenging activity of standard ascorbic acid given in respective tables and figures. Among all the extracts of leaves and fruits it was observed that methanolic extract of fruit shows the maximum scavenging activity with IC50 38µg/ml. The comparison of IC50 values of fruit and leaves along with standard is shown in respective figures.

Table 4. DPPH Free radical scavenging potential of leaves and fruits of *Duchesnea indica* at 517nm Values expressed as mean ± Standard Deviation (n=3)

	Sr No	**Leave Extract with different solvents**	DPPH scavenging effect (%) at different concentration (µg/ml) ± STDEV					
			20µg/ml	**40µg/ml**	**60µg/ml**	**80µg/ml**	**100µg/ml**	**IC50 µg/ml**
	1	**Standard Ascorbic acid**	83.62±0.54	86.4±0.45	93.52±0.5	94.3±0.28	94.72±0.26	39.92±0.4
LEAVES	**2**	**Methanolic extract of D.I**	75.6±0.5	77.01±1.0	79.5±0.5	80.3±0.4	80.7±0.3	46.2±0.3
	3	**Ethanolic Extract of D.I**	23.5±0.5	52.3±0.3	65.3±0.3	73.3±0.2	82.5±0.4	54.0±0.5
FRU	**4**	**Methanolic extract of D.I**	92.2±0.3	94.5±0.5	96.3±0.5	96.8±0.7	97.4±0.5	38.0±0.8

I T	**5**	**Ethanolic Extract of D.I**	25.1±0.3	50.5±0.5	78.6±0.5	81.5±0.5	82.5±0.4	50.3±0.4

ABTS Assay

ABTS or 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) is a source of free radicles to check the antioxidant activity of various samples. It is prepared by dissolving ABTS powder in distill water and keeping it for 12 hours in dark to generate free radicles. The radicle cations of ABTS have blue-green colour which absorb light at 734nm. The radical cation is reactive against antioxidants e.g. phenolics, thiols and Vitamin. On reaction the blue-green colour of ABTS turned colourless. The standard used for this assay was Ascorbic Acid. In this the assay the IC50 value observed for standard Ascorbic acid was (39.9±0.51)µg/ml. The maximum activity was shown by the ethanolic extract of fruit of *Duchesnea indica* thus having a lowest IC50 value i.e (37.2±0.47)µg/ml. The methanolic extract of leaves also shown the great potential of scavenging with IC50 value of (37.7±0.67) which means both fruit and leaves of *Duchesnea indica* are great source of antioxidants and thus have a great medicinal importance.

Table 5. ABTS free radical scavenging activity and IC50 of Leaves and Fruits of *Duchesnea indica* **(Absorbance 734nm)**

	Sr No	**Leave Extract with different solvents**	ABTS scavenging effect (%) at different concentration (µg/ml) ± STDEV					
			20µg/ml	**40µg/ml**	**60µg/ml**	**80µg/ml**	**100µg/ml**	**IC50 µg/ml**
	1	**Standard**	89.7±0.7	90.3±0.3	91.2±0.3	92.4±0.6	93.2±0.3	39.9±0.5

LEAVES	**2**	**Methanolic extract of D.I**	93.5±0.5	94.5±0.4	95.2±0.3	96.2±0.3	99.3±0.4	37.7±0.7
	3	**Ethanolic extract of D.I**	2.4±0.3	3.2±0.3	4.5±0.4	14.4±0.4	15.2±0.3	64.9±0.7
FRUIT	**4**	**Methanolic extract of D.I**	95.5±0.5	96.1±0.2	96.7±0.3	97.0±0.3	98.6±0.6	38.5±0.5
	5	**Ethanolic extract of D.I**	96.5±0.5	97.7±0.4	98.0±0.5	98.9±0.4	99.5±0.3	37.2±0.5

Values expressed as mean ± Standard Deviation (n=3)

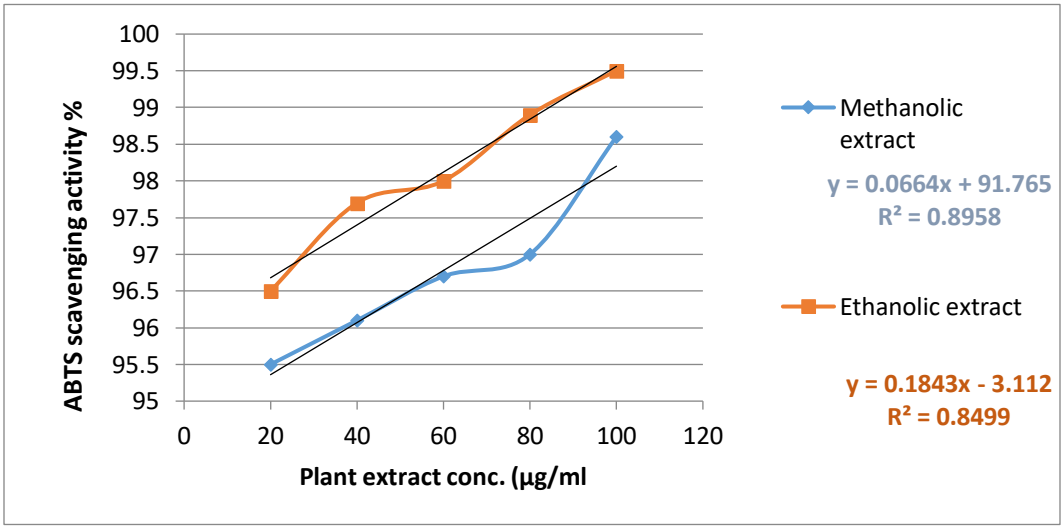

Figure 2. ABTS scavenging activity comparison Methanolic extract of leaves and Ethanolic extract of leaves

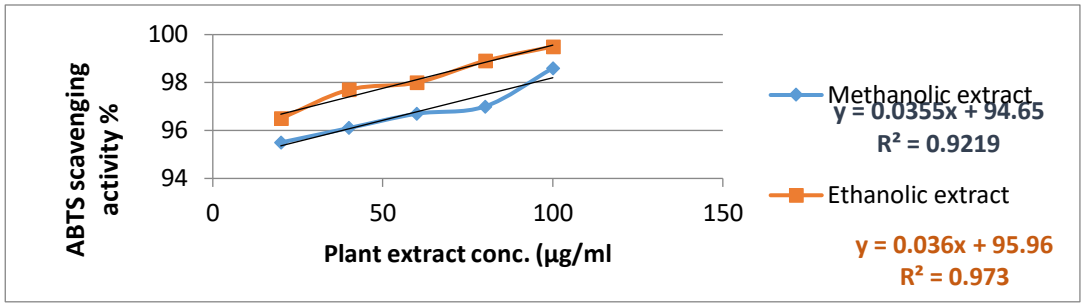

Figure 3. ABTS scavenging activity comparison Methanolic and Ethanolic fruit extracts

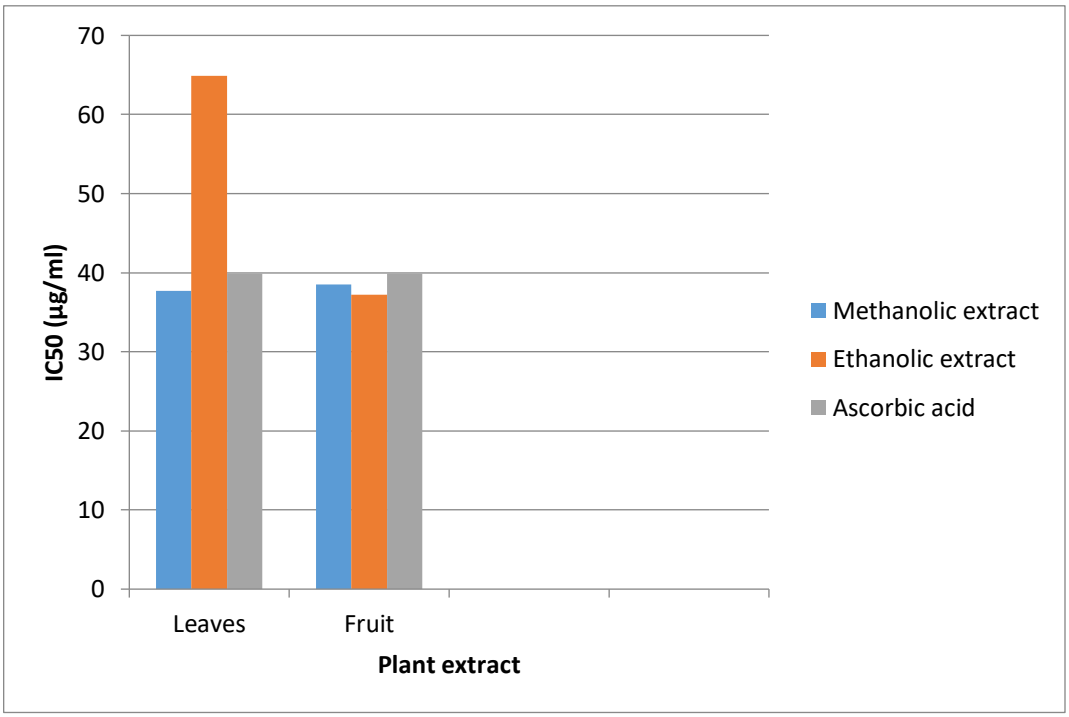

Figure 4. Comparison of IC_{50} values of ABTS assay.

Reducing power assay

In the reducing power assay presence of antioxidants in the samples would result in the reduction of Fe3+ to Fe2+. In reducing power assay absobance will increase with increase in concentration of samples in absorbance means the rate of conversion of Fe3+ to Fe2+ is increasing which means the antioxidant activity is increasing. The standard used for reducing power assay is Ascorbic acid. Test was carried out with two different extracts i.e methanolic extract and ethanolic extract. Detail values of increase in absorbance with increase in sample concentration.

Table 6. Reducing power assay of leaves and fruit extract of *Duchesnea indica* **(Absorbance 700nm)**

	Sr No	**Leaves Extract with different solvents**	Reducing power assay scavenging effect (%) at different concentration (µg/ml) ± STDEV				
			20µg/ml	**40µg/ml**	**60µg/ml**	**80µg/ml**	**100µg/ml**

	1	**Standard**	0.25±0.03	0.30±0.003	0.35±0.05	0.42±0.03	0.45±0.05
LEAVES	**2**	**Methanolic extract**	0.18±0.02	0.22±0.01	0.25±0.04	0.29±0.03	0.30±0.02
	3	**Ethanolic extract**	0.41±0.03	0.48±0.03	0.53±0.04	0.54±0.03	0.57±0.03
FRUIT	**4**	**Methanolic extract**	0.11±0.3	0.13±0.01	0.15±0.04	0.18±0.02	0.2±0.02
	5	**Ethanolic extract**	0.01±0.01	0.12±0.03	0.17±0.05	0.18±0.07	0.2±0.10

Values expressed as mean ± Standard Deviation (n=3)

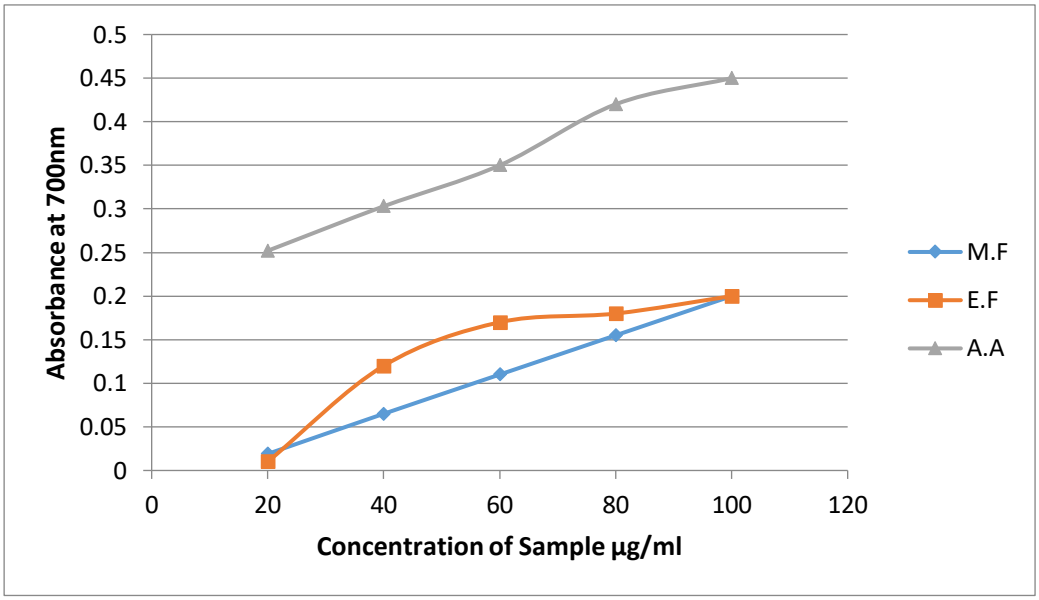

Figure 5. Reducing power of Methanolic fruit and Ethanolic fruit of *Duchesnea indica* **and Ascorbic acid as standard**

ANTIMICROBIAL ACTIVITY

Medicinal plants are commonly available and economical having less or no side effects. Most of them exhibit antimicrobial properties. Recent studies have revealed that medicinal plants from various parts of the world can provide a rich source of antibacterial activities.

Assessment of antimicrobial activities of different extracts of fruits and leaves of *Duchesnea indica* was carried out. Levofloxacin is used as standard drug.According to that ethanolic extract of leaves showed maximum activity against *Escherichia coli* inhibition zone of (10.33±5.03) mm was observed. The ethanolic extract of leaves showed no activity against *Staphylococcus aureus* and *Salmonella typhi*. Methanolic extract of leaves also showed no activity against *Pseudomonas aeruginosa* while (9±1.56)mm zone observed incase of ethanolic extract. Table 5

In case of fruit extract, maximum inhibition zone was observed in case of methanolic extract of fruit against *Pseudomonas aeruginosa* i.e. (16.82±1.28) while ethanolic extract showed no activity against same strain. Ethanolic extract of fruit also showed no activity against (*Escherichia coli*). The minimum amount of extract required to inhibit antibacterial activity is expressed as MIC µg/ml. The MIC of values of Methanolic and ethanolic extract of leaves and fruit of *Duchesnea indica* are shown in respective tables and figures.

Table 7. Antibacterial activity of leaves of *Duchesnea indica*

Test organism	**Diameter of zone of inhibition (mm)**		
	Methanolic extract of leaves 200µg/dicsc	**Ethanolic extract of leaves 200µg/disc**	**Standard drug (Levofloxacin) 200µg/disc**
Escherichia coli	7.2±0.8	10.3±5.03	20.9±0.4
Staphylococcus aureus	7.2±1.9	N.A	23.5±0.5
Pseudomonas aeruginosa	N.A	9.0±1.6	21.0±0.00
Salmonella typhi	8.8±0.8	N.A	22.0±1.5

Values expressed as mean ± Standard Deviation (n=3)

Table 8.Antibacterial activity of fruit of *Duchesnea indica*

Test organism	**Diameter of zone of inhibition (mm)**		
	Methanolic extract of fruit 200µg/dicsc	**Ethanolic extract of fruit 200µg/disc**	**Standard drug (Levofloxacin) 200µg/disc**
Escherichia coli	11.0±4.6	N.A	20.9±0.4
Staphylococcus aureus	10.7±2.5	13.6±0.9	23.5±0.5
Pseudomonas aeruginosa	16.8±1.3	N.A	21.0±0.0
Salmonella typhi	8.7±1.3	11.6±2.1	22.0±1.5

Values expressed as mean ± Standard Deviation (n=3)

Table 9. Minimum inhibitory concentration (MIC) of leaves of *Duchesnea indica*

Test Organism	**MIC value of methanolic extract (µg/ml)**	**MIC value of ethanolic extract (µg/ml)**	**MIC value of standard levofloxin (µg/ml)**
Escherichia coli	50	20	5
Staphylococcus aureus	50	N.A	3.5
Pseudomonasaeruginosa	N.A	20	5
Salmonella typhi	20	N.A	3.5

Table 10. Minimum inhibitory concentration (MIC) of fruit of *Duchesnea indica*

Test Organism	**MIC value of methanolic extract (µg/ml)**	**MIC value of ethanolic extract (µg/ml)**	**MIC value of standard levofloxin (µg/ml)**
Escherichia coli	10	N.A	5
Staphylococcus aureus	15	10	3.5
Pseudomonas aeruginosa	5	N.A	5
Salmonella typhi	20	15	3.5

Using plants to cure disease is a traditional method because many compounds active against diseases are naturally present in plants. So plants have great medicinal importance. In the present

study we discussed the medicinal importance of plant named *Duchesnea indica* or commonly known as Mock strawberry or wild strawberry. This plant have been used in different chinese traditional medicine. It can work as anticoagulant, antiseptic, depurative and febrifuge. It is also used in the treatment of boils and abscesses, eczema, ringworm, stomatitis, laryngitis, acute tonsillitis, snake and insect bites and traumatic injuries. But this is study only based on its antioxidant and antimicrobial importance. Based on this study *Duchesnea indica* is highly rich in phenolic c ompounds which shows that plant have great importance as antioxidant source. For phytochemical analysis 5g of extract of both leaves and fruit is prepared in 200ml of water. The qualitative analysis shows the presence of wide range of phytochemicals e.g.alkaloids , carbohydrates,protein etc. The quantitative analysis was done to check the presence of Alkaloids(L=5.1mg, F=4.8mg), Flavonoids (L=20.56mg, F=42.8mg), total phenols(L=84.4mg, F=517mg), tanins(L=26.56mg, F=1.22mg), Saponins(L=20.4mg, F=0.78mg)in 100g of dry extract. For antioxidant and antimicrobial analysis crude extract was prepared 10g of sample in 100 g of solvent (methanol and ethanol). The resultant mixture was analyzed for different antioxidants assay. In DDPH photometric assay the IC50 value for leaves was observed (M.E= 46.2µg)(E.E=54µg) and in fruit (M.E=38µg), (E.E= 58.3µg). Similarly, the IC50 value for ABTS assay was also observed and the in recorded as (M.E=37.7µg),(E.E=64.9µg) and in fruit (M.E=38.5),(E.E=37.2). The last assay done to check antioxidant activity was reducing power assay which also shows that plant is a good antioxidant source because of increase in absorbance with increase in concentration of sample. The result of antimicrobial activity of *duchesnea indica* revealed that plant also has great potential to work against microbial agents. The best result of leaves was observed against *Escherichia coli* giving 10.33mm zone. While activity aginst other microbial agents was also observed i.e. 7.16mm zone against *staphylococcus aureus,* 9mm zone

against *Pseudomonas aeruginosa* and 8.8mm zone against *Salmonella typhi* with minimum inhibitory concentration concentration of 20µl of sample. The fruit sample showed the best antimicrobial activity against *Pseudomonas aeruginosa* giving inhibition zone of 16.82mm, *staphylococcus aureus* 13.57, *salmonella typhi* 11.63mm and *Escherichia coli* 11mm. From this study it is concluded that *duchesnea indica* is rich source of antioxidants and thus have great medicinal importance. Enhanced investigations regarding this subject can help in designing improved antioxidant and antimicrobial compounds with better and safer activity.

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