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Nutraceutical and biological attributes of wild *Withania coagulans* fruits

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ABSTRACT

The present research reports antioxidant and antimicrobial potential of various solvent extracts, recovered from the fruits of wild *Withania coagulans*, which are wildly distributed in the Balochistan, Pakistan. The yield of crude concentrated extract (CCE) from the investigated fruits was found in the range of 1.90-4.43 g/100g dry matter. The CCEs, recovered from the fruits of *Withania coagulans*, were found to contain considerable amount of total phenolic (210-257 g/100g gallic acid equivalent) and total flavonoid contents (156-201 g/100 g catechin equivalent). The CCE, recovered from the fruits of wild *Withania coagulans,* also displayed an appreciable DPPH radical scavenging potential with IC_{50} value ranged from16-23 mg/ml and exhibited good potential to inhibit the process of peroxidation (48-68%). The highest antioxidant and antimicrobial potential against a panel of pathogenic microorganisms (bacterial and fungal strains), was exhibited by aqueous ethanol CCE (80: 20; ethyl alcohol: water) while the minimum showed by the CCE, recovered with absolute methanol. The findings of this research project showed that the fruits of wild *Withania coagulans* can be utilize as a promising source of antioxidant and antimicrobial agents for the development of functional food and nutraceuticals.

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Capsule Summary: The antioxidant and antimicrobial potential of various solvent extracts of wild *Withania coagulans* fruits which investigated using different solvents and solvent system significantly affected the bioactivities of the *Withania coagulans* fruits extracts.

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INTRODUCTION

There are different types of reactive oxygen species (ROS) which can begin the process of oxidation in various biological matrices (Wang et al., 2002). These ROS have been reported to be produced as a consequences of various oxidation process including, oxidative DNA, denaturing of protein, and lipid per-oxidation, which are associated to wide range of health disorders such as arterial sclerosis, certain types of cancers, aging and inflammation (Biglari et al., 2008). The oxidative stress caused by ROS can be prevented by effective defense systems which is linked with good quality food stuffs and age of any individual concerned (Chun et al., 2003). Therefore, the consumption of good quality diet can provide potent dietary antioxidants to offer protection against the oxidative stress caused by ROS. In order to retard the process

of oxidation in lipid molecules, a variety of plant matrices can be employed as a source of potent antioxidants.

The antioxidant agents have been documented to scavenge free radicals which were believed to mediate oxidative stress that leads to a number of ailments (Silva et al., 2007). Plant-derived food has been published as a promising source of antioxidants which are required to maintain the balance with different oxidants including in ROS in our body (Ahmad et al, 2011). Synthetic antioxidants can provide protection against free radical mediated oxidative stress; however, their use is restricted due to the perceived toxic effects on the food stuffs and its consumers (Jeong et al., 2004; Iqbal et al., 2007). Due to the availability of phenolic molecules, different plant matrices have been found to show antioxidant potential (Siddhuraju, 2003; Rababah et al., 2004; Delouee and Urooj, 2007).

According to the literature, plants are impressive source natural biologically active compounds (Ghasemzadeh et al., 2011). Due to potential utilization of secondary metabolites, particularly natural antioxidant, in food and pharmaceutical industries, there is a prompt need to explore different plants and plant residue as promising source of these potent phytochemicals (Sokmen 2006, Ahmad et al., 2016). Among secondary metabolites in fruits and vegetables, phenolics, flavonoids, saponins, tannins, alkaloids and terpenoids have been reported as potential bioactives which provide protection against chronic and degenerative diseases by exhibiting innumerable biological actions like as antioxidant and antimicrobial activities (Sandhar et al., 2011). Antioxidants compounds such as polyphenols (flavonoids, lignans, phenolic acids, anthocyanins, stilbenes, flavanols), carotene and vitamins were found to lower the oxidative stress, produced by ROS (Liu, 2014; Baiano and Del, 2015). Medicinal plants have also been used as a promising source of antimicrobial agents to provide shielding effect against pathogenic microorganisms including bacteria and fungus (Bonjar and Farrokhi, 2004).

Keeping in view these medicinal attributes, plants have been explored for the presence of different biologically active components which provide protection against various diseases. A wide range of modern drugs were derived from these medicinal plants worldwide. Therefore, the intake of plant-based diet has been reported to lower the incidence of different ailments such as neurodegenerative and cardiovascular disorders (Silberberg et al., 2005; Anastasia et al., 2007). Besides the potential source of bioactives and nutraceuticals ingredients, folk medicinal plants provide valuable nutrients intended for the regulation and maintenance of bodily functions (Taran et al., 2009).

Pakistan has a plenty of important medicinal flora including herbs and shrubs. These native medicinal plants were found to possess a variety of high-value nutrients and biologically active compounds. Due to these medicinal attributes, native plants are very popular in folk medicine. *Withania coagulans,* (known as paneer dodi), is an erect, tall, enormous, much expanded bushes (Shahid et al., 2013). It is extensively cultivated in different areas of Asia and Africa to be used in in folk medicine systems to cure stomach related issues (Mathur and Agarwal, 2011). A wide range of medicinally important compounds including flavonoids and phenolic components have been identified in it (Ahmed et al., 2005). The *Withania coagulans* fruits have been utilized as laxative, diuretic, expectorant, anthelmintic and mitigating in Pakistani traditional medication formulations (Iqbal et al., 2005; Gupta and Keshari, 2013). Various parts of *Withania coagulans* were employed in traditional medication in India as antihelminthic, laxative and used to treat ailments such as ulcers, tumors and insomnia (Kirtikar and Basu, 1935; Rasheed et al., 2012). The phytochemical screening of extracts, recovered from *W. coagulans*, exhibited the occurrence of primary and secondary metabolites (Agarwal et al., 2014).

Due to health beneficial effects of medicinal plants provokes us to appraise nutritional and antioxidant potential of fold medicinal plants. According to our knowledge, no earlier reports are available on the antioxidant and antimicrobial potential of different extracts recovered using different solvents from *Withania coagulans* cultivated in Zhob, Balochistan, Pakistan. Therefore, this project, was mainly framed to assess the antioxidant and antimicrobial potential of various solvent extracts of *Withania coagulans* so as to explore its potential to be employed as potential ingredients of pharma-nutraceuticals.

MATERIAL AND METHODS

Sampling, chemical and reagents

The ripened fruits were collected from plants of *Withania coagulans* which are wildly distributed in district Zhob, Balochistan, Pakistan. The taxonomist at department of Botany, University of Education, Lahore, Pakistan, helped us in authentication and identification of collected plant samples. The tested fruit samples were washed with distilled water to remove associated debris. Then these fruit samples were shade-dried followed by grinding it into 80 mesh size and preserved in polythene bags at -4°C till further analysis.

Linoleic acid, ammonium thiocyanate, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl, FolinCiocalteu reagent, sodium nitrite, trichloro-acetic acid, gallic acid, ferrous chloride, ferric chloride, ammonium thiocyanate, anhydrous sodium carbonate, butylated hydroxytoluene, aluminium chloride, ferrous chloride, and potassium ferricyanate, were obtained from Sigma Chemicals. Other important reagents (methanol and ethanol) of analytical grade were used in this research work which were procured from Merck chemical company (Darmstadt, Germany). The basic ingredients, used to prepare culture media to execute antimicrobial assay, were purchased from The Oxoid Ltd. (Hampshire, UK). All the chemical were used as these were received from the suppliers.

Extraction of phytochemicals

Finely ground sample (10 g) was extracted using 100 ml of different solvents such as absolute methanol, aqueous methanol (80:20; methanol and water), absolute ethanol, aqueous ethanol (80:20; methanol and water), using orbital shaker (Gallenkamp, UK) for 8 hours at room temperature. Filter paper (Whatman No. 1) is used to separate the insoluble residues from solvent extract. The residues were re-extracted twice with fresh aliquots of same solvent to get maximum soluble component. The resultant extracts of same solvent were pooled. The pooled extracted were processed on rotary evaporator ((N-N series, Rikakikai Co. Ltd, Tokyo, Japan) to remove excessive solvents under vacuum at 45 °C. the crude concentrated extracts were preserved at -4 °C for further analyses.

Antioxidant potential

Total phenolics (TP)

The content of TP in the ripened fruits of wild olives were estimated following a method described by Ahmad et al., (2011). Concisely, CCEs/PRFs (1mg/mL) was diluted in deionized water (7.5 mL), mixed with Folin-Ciocalteu reagent (0.5 mL) and sodium carbonate (20% (w/v); 1.5 mL) followed by heating at 40°C for 20 min using water bath. The absorbance of final solution was taken at 755 nm using a spectrophotometer (Hitachi U-2001, model 121-0032). The TP content was presented as Gallic Acid Equivalents (GAE) g/100 g dry matter using gallic acid standard curve.

Total flavonoids (TF)

The amount of TF in the ripened fruits of wild olive was quantified following a protocol described by Ahmad et al., (2011). Briefly, CCEs/PRFs (100mg/mL) was mixed with $NaNO_2$ (5%; 0.3 mL) in the presence of distilled water (5.0 mL). After incubation, resulting solution was allowed to react with $AlCl_3$ (10%; 0.6 mL) and NaOH (1.0 *M*; 2 mL). The absorbance of final mixture was recorded at 510 nm. The concentration of TF was estimated as catechin equivalents (CE) g/100 g dry matter using catechin calibration curve.

DPPH) radical scavenging

This analysis was conducted following a protocol (Tepe et al., 2005) to evaluate the potential of CCEs/PRFs, recovered from ripened fruits of wildly grown olive, to inhibit DPPH radical in terms of IC_{50} value. The methanolic solution of DPPH (0.004%; 5mL) was mixed with each concentration (0.10 to 5.0 mg/mL) and followed by incubation for 30 min in ambient conditions. Optical density (OD) of each solution was recorded at 517 nm (Eq. 1).

$$I(\%) = \left[\frac{A_b}{A_s}\right] \times 100 \qquad (1)$$

Where, A_b and A_s are absorbance values of blank and sample, respectively, while I is inhibition.

Reducing power

Reducing power of CCEs/PRFs were assessed using methodology presented by Yen et al. (2000). Concisely, equal volume of potassium ferricyanide (1.0%), CCEs/PRFs (5-20 mg) and sodium phosphate buffer (0.2 *M*, pH 6.6) were mixed and allowed to incubate at 50°C for 20 min. Then trichloroacetic acid (10%; 5 mL) was added to the resultant solution which was spun (980 *g* ;10 min) using centrifuge machine (CHM-17; Kokusan Denki, Tokyo, Japan). The supernatant (2.5 mL) was recovered and then mixed with ferric chloride (0.1%; 0.5 mL) and distilled water (2.5 mL). The OD of the final reaction mixture was recorded at 700 nm using spectrophotometer (Hitachi U-2001).

Inhibition of peroxidation

The potential of various CCEs/PRFs was measured by inhibiting peroxidation in linoleic acid following a protocol described by Ahmad et al., (2011). Sample solution was prepared by mixing CCEs/PRFs (5 mg) with sodium phosphate buffer (0.2 *M*; pH=7; 10.0 mL), ethanol (99.8%; 10 mL) and linoleic acid (0.13 mL) followed by addition of distilled water (5mL) and incubation at 40°C. The method developed by Yen et al., (2000) was used to measure degree of oxidation. Concisely, ammonium thiocyanate (30%; 0.2 mL), ethanol (75%;10 mL), ferrous chloride (20 m*M* in 3.5% HCl; 0.2 mL) and sample solution (0.2mL) were mixed and its OD was taken at 500 nm (Eq. 2).

$$I(\%) = 100 - \left[\frac{A_s}{A_c}\right] - 100 \qquad (2)$$

Where, I (%) is a percentage inhibition, A_s and A_c are absorbance values of sample and control (treatment without CCE/PRF) at 350 h. Positive controls: ascorbic acid and butylated hydroxytoluene.

Antimicrobial activity

The antimicrobial potential of CCEs/PRFs was determined against a set of pathogenic microbial strains (*Escerichia coli, Bacillus cereus, Fusarium oxysporum, Aspergilus flavus, Staphylococcus aureus* and *Aspergilus niger*) following disc diffusion and micro dilution broth methods (NCCLS, 1999, 1997). Fungal and bacterial strains were grown at 29±2 °C and 37±2 °C on potato dextrose agar (PDA) and nutrient agar (NA)(Oxoid), respectively. Briefly, broth culture (100µL) of bacterial cell (with 10^8 cfu/mL) and fungal strains (10^4 cfu/mL spores) were spread on their respective growth media. Filter paper discs (6mm in diameter) were autoclaved and then placed on microbial cultured plates after soaking in CCEs/PRFs (100mg/mL). Negative control (without sample) and positive control

(rifamycin and fluconazole) were also run under same experimental conditions. Sample and control plates were processed for incubation at 29 ^{0}C for 48h for fungal and 37 ^{0}C for 24h for bacterial strains. The inhibition of tested microbial growth was appraised by measuring the zone of inhibition (mm) against the control to express antimicrobial potential.

Minimal inhibitory concentration (MIC) was calculated by culturing bacterial and fungal strains in nutrient broth (NB) and sabouraud dextrose broth (SDB) augmented with Tween 80. Positive controls (tested microorganisms without extracts), sterility control (Tween 80 + test extract + NB) and growth control (Tween 80 + NB) were also run under similar experimental conditions. Rifamycin and fluconazole were employed as standard bactericidal and fungicidal compounds, respectively. After incubation, the lowest concentration of CCE/PRF without growth was recorded as MIC value.

RESULTS AND DISCUSSION

Extracts yield

The extraction yield (g/100g of dry matter) from the fruits of wild *W. coagulans* using different extracting solvent systems (methanol (100%), aqueous methanol (80:20, methanol: water), ethanol (100%), aqueous ethanol (80:20, ethanol: water) and distilled water (Table 2). The fruit extracts contain antioxidative components which are dependent on the solvents used for their recovery. The yield of crude concentrated extract was found to range 1.906g-4.430 g/100g of dry sample weight. The maximum yield (4.430 g/100 g of dry weight) was recorded with distilled water, while (1.906 g/100 g of dry weight) was estimated as the minimum yield recovered from the fruits of wild *W. coagulans* using methanol (100%). The highest yield of concentrated crude extracts from wild *W. coagulans* fruits with distilled water showed its better efficiency to recover antioxidant components. The recovery of highest extraction yield using distilled water was found to be in line with results reported by Oktay *et al*. (2003). The findings of this research project exhibited a significant ($p < 0.05$) variation in the yield of extract with respect to solvent systems used. Ahmad et al. (2009) reported that extraction yield (14.17g/100g) obtained from the fruits of *W. coagulans* using ethanol (90%) was found to be higher than the findings of present study. The variation in the extraction yield may be attributed to biotic and abiotic conditions such as soil nature, climatic conditions of the plant habitat and diversity of extraction systems. The extracted amount of antioxidants yield is dependent on the nature of plant to be extracted, amount of extracting

Table 1: Extraction yield, total phenolic and total flavonoid content in CCEs of *Withania coagulans* fruits

Solvents	Yield (g/100 g of dry weight)	Total phenolics (GAE g/100 g of dry matter)	Total flavonoids (CE g/100 g of dry matter)
Absolute methanol	1.906±0.04	210±5.2	156±6.2
Aqueous methanol	2.871±0.08	221 ±3.3	178±3.5
Absolute ethanol	2.313±0.09	235±6.7	187±5.5
Aqueous ethanol	2.116±0.03	257 ±7.1	201±2.1
Absolute acetone	23.10±1.03^{f}	0.79±0.11^{d}	0.08±0.0^{d}
Aqueous acetone	27.25±1.43^{e}	1.74±0.17^{bc}	0.12±0.03^{c}
Distilled Water	4.430±0.14	234 ±8.9	197±4.0

Values (mean ± SD) are average of three replicates, analyzed individually. Superscript letters in a same column display significant differences (p< 0.05) of means among the extracting solvents

Table 2: Reducing power, DPPH radical scavenging, Inhibition of linoleic acid peroxidation of CCE of *Withania coagulans* fruits

Solvents	Reducing Power (λ = 700 nm)				IC_{50} value	PI*
	10 mg	20 mg	30 mg	40 mg	(mg/mL)	(%)
Absolute methanol	0.07±0.03	0.17±0.02	0.28±0.02	0.34±0.06	23±0.3	48±0.9
Aqueous methanol	0.11±0.05	0.21±0.07	0.32±0.01	0.38±0.06	20±0.5	55±1.7
Absolute ethanol	0.09±0.04	0.26±0.06	0.37±0.03	0.45±0.08	21±0.4	57±2.4
Aqueous ethanol	0.14±0.05	0.30±0.07	0.39±0.04	0.53±0.1	17±0.7	68±1.3
Absolute acetone	0.22±0.01	0.35±0.03	0.59±0.05	0.76±0.03	0.78±0.07a	41.83±3.28c
Aqueous acetone	0.36±0.02	0.61±0.02	0.96±0.02	1.05±0.02	0.49±0.04b	47.01±2.86c
Distilled Water	0.12±0.04	0.19±0.06	0.31±0.09	0.39±0.02	16±0.6	59±2.1

*PI = Peroxidation inhibition

solvent and nature as well as the techniques of extraction used (Hsu *et al.* 2006). An efficient extraction technique is beneficial to recover maximum amount of potent bioactive compounds from plant material.

Total phenolic and flavonoid content

Due to the presence of potent bioactive compounds like phenolic and flavonoids, plants have extensively been utilized in food industry to retard the process of oxidation in food matrices (Aneta *et al*. 2007). These potent natural phenolic antioxidants are recovered from different parts of plants like roots, shoot, leaves, fruits, seeds and flowers (Awika *et al*. 2003); Katalinic *et al*. 2006). Therefore, screening of different plants including herbs and shrubs as a viable source of flavonoid and phenolic components is very essential. However, an effective extraction system should be employed to recover considerable amounts of bioactives.

Total flavonoids and phenolic components were extracted from *Withania coagulans* fruits using different solvent systems (Table 1). Total phenolics and flavonoids isolated from the tested fruits using different solvent systems ranges from 210-257 GAE (g/100g of dried sample) & 156-201 CE (g/100g of dried sample), respectively. Among tested extracting solvents, aqueous ethanol recovered maximum total phenolic content (257 g/100g GAE) and total flavonoid contents (201 g/100g CE), while, the minimum TPC (210 g/100g GAE) and TFC (156 g/100g CE) was extracted using methanol (100%). The amount of TP and TF, recovered from tested fruits using different solvent systems were found to be varied significantly ($p < 0.05$) among different solvent systems used. This variation in TPC and TFC were reported to linked to the ability of the extracting solvents to extract intrinsic antioxidant components from fruits of *Withania coagulans.* Due to better extraction power and reduced toxicity, ethanol is preferred to recover potent phenolic antioxidant components from plant matrices under investigation (Karaden et al., 2005; Tung et al., 2007). According to Jaffery *et al*. (2003) that the variation in the amount of recovered TPC and TFC is associated with different factors including habitat condition, maturity level at time of harvesting and soil conditions. To the best of our knowledge, no earlier reports are available on the recovery of TPC and TFC from the fruits of wild *Withania coagulans* with which we could compare the results of our present analysis.

DPPH radical scavenging

DPPH has been documented as stable free radical of violet color that exhibit absorption maxima in the range 515-528 nm. This free radical is protonated by phenolic components and imparts yellow color by losing its chromophore. Antioxidant potential of plant extract (by scavenging DPPH

Table 3: Antibacterial activity of CCEs recovered from *Withania coagulans* fruits

Solvents	Zone of inhibition (mm)			MIC (mg/mL)		
	S. aureus	*E. coli*	*B. fumilus*	*S. aureus*	*E. coli*	*B. fumilus*
Absolute Methanol	10.0±0.5^{d}	11±0.26	13±0.40	31±0.62^{b}	28±1.05	25±0.60
Aqueous Methanol	15.0±0.8^{bc}	12±0.24	15±0.45	30±0.80^{c}	27±0.90	24±0.82
Absolute ethanol	12.0±0.8^{c}	12±0.41	16±0.34	27±0.74^{c}	26±0.88	23±0.56
Aqueous ethanol	16.0±0.6^{b}	13±0.36	18±0.28	24±0.65^{d}	23±0.63	22±0.32
Absolute acetone	8.0±0.6^{e}	7.0±0.2^{f}	6.0±0.2^{e}	32±0.54^{a}	36±10^{a}	34±12^{a}
Aqueous acetone	10.0±0.4^{cd}	10.0±0.4^{e}	7.0±0.2^{e}	30±0.82^{b}	31±8^{b}	29±10^{ab}
Distilled water	11±0.5	11±0.44	15±0.55	28±0.2	25± 0.35	24±0.58
Amoxicillin	24^{a}	26^{a}	18^{a}	-	16^{b}±0.17	18^{b}±0.19

Values (mean ± SD) are average of three replicates, analyzed individually. Superscript letters in a same column display significant differences ($p< 0.05$) of means among the extracting solvents.

Table 4: Antifungal activity of CCE recovered from *Withania coagulans* fruits

Solvents	Zone of inhibition (mm)			MIC (mg/mL)		
	A. flavus	*A. niger*	*F. oxysporum*	*A. flavus*	*A. niger*	*F. oxysporum*
Absolute methanol	6±0.6^{d}	7±0.24	5±0.30	46±0.7^{c}	38±0.86	36±1.07
Aqueous ethanol	7±0.7^{b}	8±0.08	7±0.31	43±0.8^{e}	35±0.45	33±1.22
Absolute ethanol	6±0.7$_{b}$^{c}	8±0.22	8±0.34	45±8^{cd}	36±0.82	33±0.89
Aqueous ethanol	8±0.6^{b}	11±0.12	9±0.28	34±0.10^{d}	34±0.24	30±0.70
Absolute acetone	5±0.3^{f}	6±0.35	5±0.24	49±0.11^{a}	42±0.85	43±0.74
Aqueous acetone	6±0.3^{e}	8±0.41	7±0.51	39±0.10^{b}	41±0.54	39±0.68
Distilled water	7±0.50	8±0.16	6±0.28	38±0.11	36±0.46	33±0.84
Flumequine	24^{a}	28^{a}±0.71	24^{a}±0.86	29±0.15	21^{b}±0.15	25^{b}±0.19

Values (mean ± SD) are average of three replicates, analyzed individually. Superscript letters in a same column display significant

free radical) increases with the higher concentration of phenolic components in it (Sanchez-Moreno et al., 1999).

Fruit extracts of wild *Withania coagulans* have been found to exhibit considerable free radical scavenging potential, with IC_{50} values (the concentration of plant extract that scavenged 50% of DPPH free radicals) varies over the range 16-23 mg/ml (Table 2). The fruit extract recovered with distilled water exhibited the lowest IC_{50} value (16mg/mL) while the maximum value of IC_{50} was estimated for methanol extract. Among other extracts, methanolic extract exhibited minimum DPPH radical scavenging activity (23mg/mL) which was attributed to the lower number of phenolic components. When the DPPH radical scavenging activity of all tested extracts were compared and found lesser than that of butylated hydroxy toluene (BTH). This DPPH radical scavenging capacity of plant extracts was associated with existence of phenolic contents in it (Siddhuraju et al., 2002). There are no previous reports available related to the DPPH radical scavenging activity of *Withania coagulans* fruit extract with which our current findings can be compared.

Antioxidant activity in linoleic acid system

Linoleic acid an unsaturated fatty acid when oxidized it produces peroxides and that further oxidizeFe^{2+} to Fe^{3+}, and then formation of complex along SCN^-, At 500 nm absorbance is measured by spectrophotometer to check the concentration. A higher concentration of peroxides formed during reaction. Greater absorbance value is obtained due to large concentration of peroxide which denotes the lesser antioxidant potential.

The potential of CCE, recovered from the fruits of wild *Withania coagulans,* to inhibit lipid peroxidation, is estimated to which indicates their antioxidant activity (Table 2). For absolute methanol and aqueous methanol (80:20, methanol: water), the range of inhibition of linoleic acid oxidation were found to be 48% and 55% respectively; and 57% and 68**%** for absolute ethanol and aqueous ethanol (80:20, ethanol: water); while for distilled water it was recorded as 59% inhibition of linoleic acid oxidation. The fruits extract recovered using aqueous ethanol (80:20, ethanol: water) displayed significantly ($p < 0.05$) higher inhibition of peroxidation (68%) as compared to other extracts that is ascribed the occurrence of larger amount of phenolic ingredients in it. All the tested crude extracts of *Withania coagulans* fruit exhibited lower inhibition of linoleic acid oxidation when correlated with synthetic antioxidants BHT (92.01%). We are unable to compare the current findings of this assay with literature as there is no report available on percentage inhibition of linoleic acid oxidation for extracts of *Withania coagulans* fruit.

Reducing power

In different solvent extracts, recovered from tested plant, the extent of reducing power is also used to indicate the antioxidant activity. In this test, the ferric ions (yellow color) are reduced to ferrous ions (bluish green color). This reducing potential of fruit extracts are directly dependent on the color intensity, due to presence of antioxidant component. If intensity of color is greater, absorption will be higher; therefore, antioxidant potential will be higher (Zou et al., 2004). Siddhuraju *et al*. (2002) and Ahmad et al. (2011) reported that antioxidant potential is directly linked with reducing potential of bioactive compounds. In current assay, all the tested extracts showed a consistent rise in reducing potential when the concentration of extracts was enhanced (Table 2). Fruit extracts with concentrations (10-40 mg/mL) were evaluated for their reducing potential. The resultant absorbance recorded was found over the range 0.11–0.53. Absorbance value (0.14-0.53) was recorded as the maximum for the fruit extract recovered with aqueous ethanol (80: 20, ethanol: water), while (0.07-0.34) was recorded minimum for methanol (100%) fruit extract. We are unable to compare the results of this assay with literature as no report is available in literature.

Antimicrobial activity

The crude concentrated extracts, recovered from the fruits of wild *Withania coagulans,* showed good antimicrobial activity against a set of pathogenic microbes (Table 3). Disc diffusion method was used to record antimicrobial activity, moreover, minimum inhibitory concentration was also calculated using micro-dilution broth method. The tested fruit extracts showed larger zone of inhibition for *Bacillus pumilus* ranging from 13-18 mm and least MIC values (22-34) mg/mL which accounts as the most sensitive microorganism. Fruits extracts of investigated plant exhibited good antimicrobial potential against *Escherichia coli* and *S. aureus* with smaller inhibition zones ranging from 11– 13 mm, 8-16mm and higher MIC values, 23-36 mg/mL and 24-32mg/ml, respectively. The tested CCES displayed the higher inhibition zones against *A. niger* ranging from 6-11 mm with lower crossponding MIC value varied over the range of 35-42 mg/ml and found to be more sensitive fungal strain (Table 4). So, these CCEs showed superior antifungal potential when compared with *F. Oxysporum* and *A. flavus* that showed zone of inhibition ranging from 5-9 mm and 5-8mm with crossponding MIC value ranging from 3343 mg/mL and 34-49 mg/ml, respectively. Generally, the *Withania coagulans* fruit extracts exhibit good antifungal potential. Previous studies (Celiktas et al., 2007) revealed that extracts having modification in chemical composition may alter the biological actions. This diversity can be explained in term that plants have a huge variety of antimicrobial agent accumulated in different portion.

CONCLUSIONS

The phenolic antioxidant components have been found very important to maintain good health and to provide shielding

effect to human body against various ailments including aging, cardiovascular diseases and certain types of cancer. Different solvent extracts, recovered from fruits of *Withania coagulans*, have been evaluated for antioxidant and antimicrobial attributes. The yield of CCEs were found to contain over the range of 1.90 to 4.43 g/100g dry matter. Considerable amount of TPC (2.30–2.76 g/100g as Gallic acid equivalent) and TFC (1.76–2.35 g/100g as catechin equivalent) were detected in CCEs. A regular surge in reducing potential was recorded with increasing concentration of CCEs. The CCEs were also evaluated to inhibit the process of peroxidation which were found to vary over the range of 48% to 68%. The CCEs exhibited remarkable potential to scavenge the free radicals with IC_{50} value ranges from 16 to 23 mg/ml. When these CCEs of tested wild fruits were investigated against a panel of pathogenic microorganisms, they showed excellent ability to inhibit the growth of microorganisms. Among other tested microorganisms, *Bacillus pumilus* was found to be the most sensitive bacterial strains with biggest zone of inhibition (13 to 18mm) and smallest MIC values (23 to 25 mg/mL) with aqueous ethyl alcohol CCE of tested wild fruits. The findings of this research project showed that the fruits of wildly distributed *Withania coagulans* could be explored as potential candidate to be used in the development of functional food and nutra-pharmaceuticals.

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