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Antioxidant And Antimicrobial Activities Of Essential Oil And Extracts Of Viburnum Nervosum Growing Wild In The State Of Jammu And Kashmir

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Abstract:
The present study was conducted to examine the antioxidant and antimicrobial activities of essential oil extracts from roots of Viburnum nervosum (guch) collected from Sudhan Galli, District Muzaffarabad, Azad Jammu & Kashmir at an altitude of 7000 feet. The essential oil yields from this plant were found to be 0.025 % (w/w). GC and GC–MS analysis of the guch essential oil revealed the presence of 23 components, of which 15 were identified, representing 98% of the essential oil. The eudesmol (30.34%), caryophyllene oxide (17%), linalool (12.60%), spathulenol (10.70%), and ledene (9.80%) were the major components. The guch extracts contained appreciable level of total phenolic contents (628.26-968.04 GAE, mg/100g), total flavonoid contents (376.75-682.87 CE, mg/100g). Essential oil also exhibited good DPPH radical scavenging activity showing IC_{50} 33.32, 25.65, 23.07, 25.13, 22.97 μg/mL, and inhibition of peroxidation 19.23 and 93.04%, respectively. Of the Viburnum nervosum essential oil and extracts tested 80% ethanol exhibited maximum antioxidant activity. Essential oil showed appreciable mild antimicrobial activity against selected strains of bacteria and pathogenic fungi, as assessed by disc diffusion assay. The antioxidant activity of essential oil and extracts of demonstrated significant ($p < 0.05$) variations in results.

Key words*:* *Viburnum nervosum, essential oil, phenolics, antimicrobial*

1.Introduction

Reactive oxygen species (ROS), nitrogen reactive species (NRS) are inhibited by antioxidant and prevent lipid peroxidation and other free radical-mediated processes and are able to protect cell membrane as well as processed foods from oxidative damage attributed to the reaction of free radicals. The use of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) in foods is dispirited due to their professed carcinogenic risks and safety concerns. During the couple of decade, great effort has been dedicated exploring safer natural antioxidants from diverse sources [1, 2].

So, the use of plant-based antioxidants such as of phenolic substances like flavonoids and phenolic acids and tocopherols in foods, as well as preventive and therapeutic medicine is gaining much attention and used as nutraceutical. Such natural substances are believed to exhibit anticarcinogenic, antimutagenic activities and offer assorted health-promoting effects because of their antioxidant potential. [3, 4].

The genus viburnum comprises about 300 species, distributed in temperate and subtropical regions of Asia, North America and Malaysia of which six species exist Northern Pakistan and in the State of Jammu and Kashmir [5, 6].

Viburnum nervosum (Caprifoliaceae), locally known as 'Guch', is used as an astringent and emmenagogue in Azad Kashmir [7]. It yields an essential oil with potential perfumery applications. In Kashmir, its roots are used in the treatment of acute furunculosis [8]. Several known triterpenes, alcohols and flavones have been isolated from the plant [9].

A large deciduous precocious shrub, 2-3 m tall with stiff stout branches; winter buds protected by scales. Leaves 5-12 x 2.5-6 cm, elliptic, oblong, acute, sharply toothed, cuneate at the base, more or less hairy on the nerves. Main lateral nerves 7-10 pairs, prominent, closely parallel, undivided. Flowers in terminal sessile corymbs. Bracts variable in size, hairy. Calyx tube mm long, glabrous; lobes short, ciliate. Corolla long-tubular, 10-15 mm lobes rounded, spreading, white to rose-pink. Stamens in two series, 2 attached near the mouth of corolla tube, 3 lower down, anthers included. Stigma subsessile, 3-lobed. Drupe c. 1 cm long, ellipsoid, compressed, black when ripe. Seed sigh grooved on one side, deeply grooved on the other with incurved margins. One of the commonest shrubs in the Kashmir between 1500-3000 m. The flowers often appear soon after the leaves have fallen in November and continue to appear till June. The flowers that appear in early winter before snow are smaller and in much denser cymes than the

flowers which appear in late spring. The flowers are sweet scented, but leaves emit a bad smell when bruised. The fruit is sweetish and edible [5, 6].

Pharmacognostical studies were carried out on the roots of *viburnum nervosum*, roots contain fixed oil and other minor constituents like tannins and sugar. Roots obtained from Kashmir are in general richer in essential oil contents than roots obtained from other parts of the world [10] .

Viburnum nervosum (Capprifoliaceae) is used in the Indian system of medicine as astringent and emmenagogue. It yields as essential oil, which is used as a potential perfumery material. In Kashmir its roots are used in treatment of acute furunculosis. several triterpenes, alcohols and flavones have been isolated from this plant. From the roots bergenin was isolated and has been found to possess use in the treatment of hypercholestraemia, kidney stones, fever, diarrhoea, pulmonary infection, as an antioxidant and anticancer agents [11-14].

The essential oil is valued in high class perfumery and cosmetics where it is used for blending purpose. The essential oil has strong antiseptic and disinfectant properties. It has marked carminative properties injection of the essential oil produces vasodilatation. The roots owe its insecticidal properties to its essential oil contents [15]

The aim of the present study was to evaluate the comprehensive antimicrobial and antioxidant potential of the essential oil, methanol and ethanol extracts of guch root grown in Sudhan Galli, District Muzaffarabad, Azad Jammu and Kashmir.

2.Materials And Methods

2.1.Collection And Pretreatment Of Plant Material

The fresh roots of *Viburnum nervosum* were collected and oven dried in August 2010 from Sudhan Galli, District Muzaffarabad, Azad Jammu & Kashmir. The plant was identified and authenticated by the plant taxonomist, Prof. Shafique-ur-Rehman, AJKBOT-431**,** at the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad. Collected specimens were dried at 35 C in a hot air oven (IM-30 Irmec, Germany) and stored in polyethylene bags at -4 °C till for further analysis.

2.2.Chemicals And Reagents Used In The Analysis

Linoleic acid, 2, 2,-diphenyl-1-picrylhydrazyl, gallic acid, Folin-Ciocalteu reagent, ascorbic acid, trichloro-acetic acid, sodium nitrite, aluminum chloride, ammonium

thiocyanate, ferous chloride, feric chloride, potassium fericyanate, butylated hydroxytoluene (99.0 %), homologous series of C_9-C_{24} *n*-alkanes and various references from obtained from Sigma Aldrich Chemical Co. (St Louis, MO, USA). All other chemicals (analytical grade) i.e. anhydrous sodium carbonate ferrous chloride, ammonium thiocyanate, chloroform and methanol used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise. All culture media and standard antibiotic discs were purchased from Oxoid, Hampshire, UK.

2.3.Extraction And Isolation Procedure

2.3.1.Hydrodistillation Method

Oven-dried roots of Viburnum nervosum (2 K g) was ground and subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus as recommended by British Pharmacopeia (1988). Briefly, the plant was immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapors and finally collected in a condenser. The distillate was isolated and dried over anhydrous sodium sulfate [16].

Ground (80-mesh) plant sample (20g) was extracted separately with 200 mL of 100% methanol, 80% methanol (80:20, methanol:water, v/v), 100% ethanol, 80% ethanol (80:20, ethanol:water, v/v) using an orbital shaker (Gallenkamp, UK) for 8 hour at room temperature at 120 rmp. The extracts were separated from solids by filtering through Whatman No. 1 filter paper. The remaining residue was re-extracted twice and extracts were pooled. The solvent was removed under vacuum at 45 °C, using a rotary vacuum evaporator (N-N Series, Eyela, Rikakikai Co. Ltd., Tokyo, Japan) and stored at -4 ^{o}C until used for further analyses.

3.GC/MS Analysis

The oil was analyzed by GC-MS using a Hewlett-Packed GC MSD 5890 Series 2 mass spectrometer (70eV) on a SE-30 column (25m x 0.25 mm) was used with helium as a carrier gas (1.8 mL/min). GC oven temperature was kept at 50˚C for 2 minutes and programmed to 250˚C at a rate of 5˚C/min, then kept constant at 250˚C for 2 minutes and then programmed to 250˚C at a rate of 1˚C/min. split ratio was adjusted at 50:1. The injector and detector temperatures were at 260˚C. MS were taken at 70 eV. Mass range was from 35 to 425 m/z.

4.Compounds Identification

The identification of components was based on comparison of their mass spectra with those of NIST mass spectral library [17] and those described by [18] as well as on comparison of their retention indices either with those of authentic compounds or with literature values.

The identification of the oil constituents was based on a comparison of their retention indices relative to (C_9-C_{24}) *n*-alkanes either with those of published data or with authentic compounds. Compounds were also identified using their MS data compared to those from the NIST mass spectral library.

5.Antimicrobial Activities

5.1.Microbial Strains

The essential oil and extract of Viburnum nervosum was individually tested against a set of selected microorganisms, including Gram-positive bacteria: Staphylococcus aureus (S. aureus), API Staph TAC 6736152, Gram-negative bacteria: Escherichia coli (E. coli) ATCC 25922, and four pathogenic fungi, Aspergillus niger ATCC 10575 (A. niger), Fusarium solani (F. solani), and Rhizopus solani (R. solani). The pure bacterial and fungal strains were obtained from Department of Clinical Medicine and Surgery and were verified by the Department of Veterinary Microbiology, Government of Azad Jammu and Kashmir, Pakistan. Bacterial strains were cultured overnight at 37 ºC in Nutrient agar for 2 h (Oxoid, Uk) while fungal strains were cultured overnight at 28 ºC using Potato dextrose agar for 4 h (PDA, Oxoid, England).

5.2.Disc Diffusion Method

The antimicrobial activity of the essential oil and extract, were determined by microbiological assay disc diffusion method. Briefly, 100 µL of suspension of tested microorganisms, containing 10^7 colony-forming units (CFU)/mL of bacteria cells and 10^5 spores/mL of fungi spread on NA and PDA medium, respectively. The filter discs (6 mm in diameter) were individually impregnated with 15 µL of sample, placed with the help sterilized forceps on the agar plates which had previously been inoculated with the tested microorganisms. Discs without samples were used as a negative control. Rifampicin (30 µg/dish) (Oxoid, England) and Fluconazole (25 µg/disk) (Oxoid, England) were used as positive reference for bacteria and fungi, respectively to compare

sensitivity of strain/isolate in analyzed microbial species. Plates, after 2 h at 4 ºC, were incubated at 37 ºC for 24 h for bacteria and at 28 ºC for 48 h for fungal strains. Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones (zone reader MAS-GmbH, Hessen, Germany) in millimeters for the organisms and comparing to the controls.

5.3.Determination Of Minimum Inhibitory Concentration

For the determination of minimum inhibitory concentration (MIC), which represents the concentration that completely inhibit the growth of microorganisms, a micro-dilution broth susceptibility assay was used, as recommended by Clinical Laboratory Standards Institute (CLSI, 2007). All tests were performed in nutrient broth (NB, Oxoid, England) for bacterial and sabouraud dextrose broth (SDB, Oxoid, England) for fungal strains supplemented with Tween 80 detergent to a final concentration of 0.5 % (v/v). Bacterial strains were cultured overnight at 37 ºC in NB and the fungi were cultured overnight at 28 ºC in SDB. Dilutions series were prepared from 0.03 to 72.0 mg/mL of the compounds in a 96-well microtiter palte, 160µL of NB and SDB for bacteria and fungi, respectively were added onto microplates and 20 µL of tested solution. Then, 20 µL of 5 x 10^5 CFU/mL of standard microorganism suspension were inoculated onto microplates. Plates were incubated at 37 ºC for 24 h for bacteria, and at 28 ºC for 48 h for fungi. The same test was performed simultaneously for the growth control (NB + Tween 80) and sterility control (NB + Tween 80 + test sample). Rifampicin 30 µg was used as a reference compound for antibacterial and Fluconazole for antifungal activities. The growth was indicated by the presence of a white ‘‘pellet’’ on the 96 well bottom.

6.Antioxidant Activity

6.1.DPPH Radical Scavenging Assay

The antioxidant activity of the Viburnum nervosum oil and its extracts were assessed by measuring their scavenging abilities to 2, 2′-diphenyl-1-picrylhydrazyl stable radicals. The DPPH assay was performed as described by [19]. The samples from 0.5 to 15.5 µg/mL were mixed with 1 mL of 90 µM DPPH solution followed by addition of 95% MeOH up to final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. Synthetic antioxidant, BHT was used as a positive control. The disappearance of DPPH was read spectrophotometrically at

515 nm (Hitachi U-2001, Tokyo, Japan). Inhibition of free radical by DPPH in percent (%) was calculated in following way:

- $I (\%) = 100 \times (A_{blank} - A_{sample}/A_{blank})$

Where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test samples. IC_{50} values, which represented the concentration of essential oil that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

7.Percent Inhibition In Linoleic Acid System

The antioxidant activity of Viburnum nervosum oil and extracts were determined by using inhibition of linoleic acid oxidation, following the method described by [20] with modification. The test samples (50 µg) were mixed with 1 mL of ethanol (v/v), linoleic acid (2.5%, v/v), 99.5% ethanol (4 mL) and 4 mL of 0.05 M sodium phosphate buffer (pH 7). The solution was incubated at 40 °C for 175 hrs. The extent of oxidation was measured by peroxide value using the colorimetric method described by [20]. To 0.2 mL sample solution, 10 mL of ethanol (75%), 0.2 mL of an aqueous solution of ammonium thiocyanate (30%) and 0.2 mL of ferrous chloride solution (20 mM in 3.5% HCl) were added sequentially. After 3 min of stirring, the absorbance was measured at 500 nm, using spectrophotometer (Hitachi U-2001, Tokyo, Japan). A control was performed with linoleic acid but without samples. Butylated hydroxytoluene (BHT) was used as positive control. Percent inhibition of linoleic acid oxidation expressed as percent was calculated as follows:

% inhibition of linoleic acid oxidation = 100 – [(Abs. increase of sample at 175h/ Abs. increase of control at 175h) ×100]

7.1.Statistical Analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data were performed by Analysis of Variance (ANOVA) using STATISTIX 8.1 (Stat Soft Inc, Tulsa, OK, USA) software. A probability value of $p \leq 0.05$ was considered to denote a statistical significance difference. Data are presented as mean values ± standard deviation calculated from triplicate determinations.

8.Results And Discussion

The fresh plant material of Viburnum nervosum (2.0 Kg) was subjected to hydro-distillation for three hours. The sample afforded pale yellow to brownish colored oil with characteristic aromatic odour. The oil has been subjected to GC/MS analysis. The gas chromatogram of the oil revealed the presence of 23 components, of which 15 were identified, representing 98% of the essential oil. The major components identified from plant were Eudesmol (30.3%), Caryophyllene oxide (17.0%), Spathulenol (10.7%) and Linalool (12.65%).The constituents are shown in Table-I. Inhibitory effects of the ethanol extract of Viburnum nervosum on the growth, acid production, adhesion, and water-insoluble glucan synthesis of Streptococcus mutans (S. mutans) were examined. The growth and acid production of Streptococcus mutans were inhibited by the presence of ethanol extract of Viburnum nervosum (0.5-4 mg/mL) significantly adherence of Streptococcus mutans in a dose dependent manner.

9.Antioxidant Activity

9.1.DPPH Radical Scavenging Assay

We investigated the free radical scavenging activity and lipid oxidation inhibition of Viburnum nervosum essential oil and extracts. Free radical scavenging activities of the Viburnum nervosum essential oil and extracts were measured in DPPH assay. Free radical scavenging capacity increased with increasing extract and essential oil concentration (table 3). Viburnum nervosum essential oils and extracts showed excellent radical scavenging activity with IC_{50} values of essential oil 33.32 and 25.6-23.07 μg/ mL^{-1}, in 100 and 80% methnol respectively. While 25.13-22.97 μg/ mL^{-1} by 100% and 80% ethanol respectively. The free radical scavenging activity of ethanol extract was superior to essential oil. Furthermore, 80% ethanol and methanol extracts exhibited more scavenging activity than absolute ethanol and methanol extracts. When comparing with synthetic antioxidant BHT, both essential oil and extracts offered slightly lower antioxidant activity.

9.2.Percent Inhibition Of Linoleic Acid Oxidation

Table-3 shows the level of % inhibition of linoleic acid oxidation as exhibited by the *Viburnum nervosum* essential oil and extracts. 80% ethanol extract offered significantly ($p < 0.05$) higher inhibition of peroxidation (70.35%) than *Viburnum nervosum* essential

oil (45.05%) and other extracts (48.8 to 55.31%). When the inhibition of linoleic acid oxidation of fennel essential oil and extracts were compared with BHT, all the extracts and essential oil exhibited significantly ($p < 0.05$) lower antioxidant activity than that shown by BHT (92.1%). The order of inhibition of linoleic acid oxidation offered by essential oil and various extracts of Viburnum nervosum were as follow: BHT > 80% ethanol > 80% methanol > absolute ethanol > absolute methanol > essential oil.

Due to lack of data on % inhibition of linoleic acid oxidation of Viburnum nervosum essential oil, we could not compare results of our present reading with literature. We reported the linoleic acid peroxidation for Viburnum nervosum ranging from 40.03-69.8 % of essential oil, methanol and ethanol extracts and that of BHT equal to 93.04 %.

10.Total Phenolic And Total Flavonoid Contents

The total phenolic contents (TPC) and total flavonoid contents (TFC) of Viburnum nervosum extracts are presented in Table 4. The amount of TPC and TFC extracted from Viburnum nervosum in different solvent systems ranged from 628.26, 634.95, 528.32 and 682.87, mg/100g respectively. Methanol and Ethanol extract (80%) of the Viburnum nervosum showed the highest TPC, 634.95 and 968.04 mg $100g^{-1}$, and TFC, 456.09 and 682.87 mg $100g^{-1}$ respectively. These differences in the amount of TPC and TFC may be due to varied efficiency of the extracting solvents to dissolve endogenous compounds. The ability of different solvents to extract TPC and TFC was of the order: 80% ethanol > 80% methanol > absolute ethanol > absolute methanol. Ethanol is preferred for the extraction of antioxidant compounds mainly because of its lowers toxicity.

11.Reducing Power

The reducing potential of the tested Viburnum nervosum extracts at concentration 2.5-10 mg/mL was observed to be increased in a reducing potential with concentration. The values of absorbance recorded for the tested extracts solutions in this assay were noted to be in the range from 0.20-1.85, indicating a high correlation index ($r^2 = 0.9416$-0.9954). Maximum absorbance value (1.85) was recorded for 80% ethanol whiles the minimum for absolute methanol (0.20). The reducing power of different solvent extracts lowered in the order: 80% ethanol > 80% methanol > absolute methanol > absolute ethanol. The variations in the reducing powers of different Viburnum nervosum extracts were

statistically significant ($p < 0.05$). When these results were compared with standard ascorbic acid, all the extracts showed significant ($p < 0.05$) lesser reducing power.

12.Antimicrobial Activity

The antimicrobial activity of essential oil of Viburnum nervosum against a set of microbes like Gram positive and Gram negative bacteria and selected fungal strains is shown in Table-4. Plant material of Viburnum nervosum oil exhibited variable degree of antimicrobial activity against the all microorganism tested. The disc diffusion method for antibacterial activity showed significant reduction in bacterial growth in terms of zone of inhibition around the disc. The essential oil of Viburnum nervosum showed maximum activity against B. subtils and minimum against P. multocia. Approximately, no activity was observed by the extracts.

Results obtained from disc diffusion method, in increasing order of concentration are listed in table. The essential oil of Viburnum nervosum showing almost same activity against the antibacterial strain at high concentration. So, It was dependent this that non significant increased was observed on increase of concentration.

Viburnum nervosum oils were tested against four fungal strains; A. niger, A. flavus, Fusarium solani and R. solani. The results of antifungal activity of Viburnum nervosum oil against fungal strains are presented in Table-4. The oil exhibited antifungal activity comparable with the standard drug (Flumequinene).The sensitivity order of essential oil against selected fungal strains is A. niger > R.Solani > A. Flavus and Fusarium solani.

Viburnum nervosum essential oil shows maximum against A. Niger and R. solani and minimum against A. flavus and Fusarium solani respectively. Based on these results, it concludes that, the essential oil of Viburnum nervosum possessed broader spectrum antimicrobial activity. But the overall antimicrobial profile of Viburnum nervosum oil evidenced that this oil is not too much potent against bacterial and fungal strains.

Furthermore, the Viburnum nervosum essential oil not exhibited appreciable antimicrobial activities. The production of such essential oil and bioactive components from indigenous resources and their utilization as potential natural food preservatives could be of economic value. However, further investigations involve more detailed to antibacterial and antifungal activities in vitro and in vivo studies to establish which components of the essential oil or extracts offer the best antioxidant activity are recommended. Overall, this study presents valuable information on the composition and antioxidant attributes of Viburnum nervosum essential oil from Pakistan. It advocates its

consumption in food and pharmaceutical preparations by the local industry. In addition, Viburnum nervosum showing antioxidant activity might be explored for functional food and pharmaceutical applications, besides its traditional uses.

13.Acknowledgement

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S#	Compounds	%age composition	RI	Metho of identification
1.	Myrecine	5.4	984	RI, MS
2.	P.cymene	2.6	1025	RI, MS
3.	Linalool	12.65	1080	RI,MS,CO
4.	α-elemene	0.2	1490	RI,MS
5.	α-selinene	1.3	1493	GC-MS
6.	Elemicin	1.0	1527	RI,MS
7.	α-elemol	3.0	1549	GC-MS
8.	Spathulenol	10.7	1569	RI,MS,CO
9.	Caryophyllene oxide	17.0	1574	RI,MS,CO
10.	Globulol	0.3	1584	RI,MS
11.	α-eudesmol	30.3	1642	RI,MS,CO
12.	Ledene	9.8	1644	RI,MS,CO
13.	Glaucyl alcohol	1.0	-	GC-MS
14.	α-ionone	0.2	-	GC-MS
15.	Total	98.4		

Table 1: Percentage composition of the essential oil of Viburnum nervosum

RI: retention index on SE-30 colmun, MS: mass fragmentation, CO: co-injection with authentic samples

Compounds	Content (µg/mL) [a]
Eudesmol.	30.3
Caryophyllene oxide	17.0
Linalool	12.6
Spathulenol	10.7
Ledene	9.8

Table 2: Amounts of the major constituents in Viburnum nervosum essential oil analysis by GC - MS.

[a] Values are mean of three independent experiments.

	Essential oil	Extracts				BHT
		100% Methanol	80% Methanol	100% Ethanol	80% Ethanol	
DPPH, IC_{50} (µg/mL)	33.32 ± 0.67^{a}	25.65 ± 1.02^{b}	23.07 ± 0.35^{bc}	25.13 ± 0.85^{b}	22.97 ± 0.38^{c}	19.23 ± 0.87^{d}
Inhibition in linoleic acid system (%)	44.03 ±0 .96^{d}	47.67± 0.87^{c}	54.96 ± 1.03^{c}	51.83 ± 0.85^{c}	69.25 ± 1.67^{b}	93.04 ±1.95^{a}
Total phenolic contents^{b} (mg/100g of extracts)	----------	628.26 ± 17.35^{c}	634.95 ± 11.85^{b}	871.89 ± 19.78^{b}	968.04 ± 34.38^{a}	----------
Total flavonoid contents^{c} (mg/100g of extracts)	----------	376.75 ± 13.05^{d}	456.09 ± 15.45^{b}	528.32 ± 14.29^{c}	682.87 ±23.03^{a}	----------

Table 3: Antioxidant Activities Of Viburnum Nervosum Essential Oil, Ethanol And Methanol Extracts By Different Methods

[a]Values are mean ± standard deviation of three separate experiments.

[b]Total phenolic contents expressed as gallic acid equivalent.

[c]Total flavonoid contents expressed as catechin equivalent

Different letters in superscript indicate significant differences within solvents.

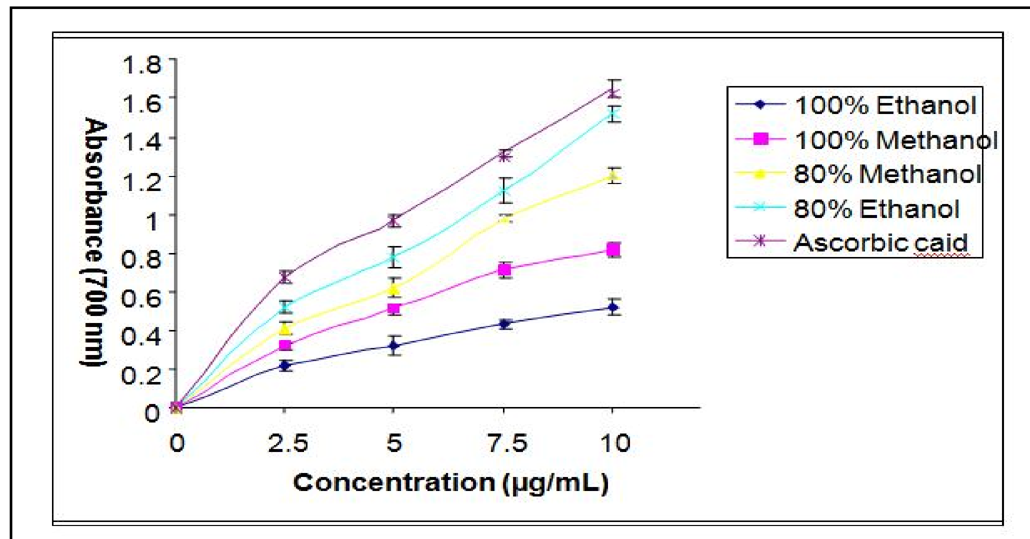

Figure 1: Reducing potential of methanol and ethanol extracts of Viburnum nervosum.

Medicinal Plant Bacterial	**B. subtils**	**S. aureus**	**E. coli**	**P. multocia**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Viburnum nervosum	13.3 ± 1.29	13.8 ± 1.65	14.1 ± 4.1	14.8 ± 1.07
Standared	25.6 ± 1.17	22.3 ± 1.36	26.23 ± 1.02	25.4 ± 1.02
Antifungal	A. niger	A. flavus	R.solani	Fusarium solani
Viburnum nervosum Standard	Mean±SD	Mean±SD	Mean±SD	Mean±SD
	13.5 ± 3.1	11.6 ± 0.62	13.8 ± 3.65	13.66 ±3.42
	13.5 ± 3.1	11.6 ± 0.62	13.8 ± 3.65	13.66 ±3.42

Table 4: Antimicrobial activities of essential oil of Viburnum nervosum against selected bacterial and fungal species.
Values are the mean ± Standard deviation of three independent experiments
No activity was recorded by ethanol and methanol extracts.

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