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Assessment of antioxidant and antibacterial activities of crude extracts of *verbena officinalis* Linn root or Atuch (Amharic)

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A B S T R A C T

Verbena officinalis Linn is a traditionally known medicinal plant which is used against a number of diseases including inflammatory conditions. In this study its antioxidant activity (reducing powers, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities), ferric reduction activity potential (FRAP), total flavonoid concentration and antimicrobial activities of 80%, 90%, 100% methanol and chloroform extracts of *V. officinalis* Linn root and 90% and100% methanol leaf extracts were determined. Its antioxidant activity increases with increase in amount of extract (10% to 40%v/v). Total flavonoid content (TFC) varied from 73.32±0.002 mgQE/100g of dry weight (90% methanol) to 42.39±0.032 mgQE/100g dry weight (chloroform), 2,2-diphenyl-1-picrylhydrazyl (DPPH), radical scavenging activity (%) was varied between 87.39% (90% methanol) to 45.57% (chloroform) while Ferric reducing antioxidant power was observed between 372.93±0.04 mgAAE/100 g extract (90% methanol) to 129.41±0.026 mgAAE/100 g chloroform in the root extract. The methanolic extract of the leaf showed less antioxidant activity than the methanolic extract of the root. Crude extracts of *V. officinalis* root showed various degree of antimicrobial activity towards drug resistance microbial pathogens. Growth inhibition tests against bacterial pathogens demonstrated concentration dependence. Moreover, gram positive bacteria were more susceptible to *V. officinalis* root extract when compared to gram negative bacteria. In general *V. officinalis* root and leave extracts possess strong antioxidant and antimicrobial activities.

Capsule Summary: Antimicrobial and antioxidant activities of the *Verbena officinalis* root extracts have been examined using different test systems. A strong correlation between antioxidant activities and TFC/TPC was observed. The results indicate that *V. officinalis* infusions could be an important dietary source of natural antioxidants with consequent health benefits.

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INTRODUCTION

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease (Scheibmeir et al., 2005). Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like

vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce health risk(Dai and Mumper, 2010; Lafay and Gil-Izquierdo, 2008). Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties (Jasuja et al., 2013). Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals including oxygen which are from a wide variety of sources are present in biological systems. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported (Kovacic and Jacintho, 2011; Sies, 1997; Ridnour et al., 2005).

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic agents. Today, natural products (and their derivatives and analogs) still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing *ca*. 25% of the total. The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. From 1983 to 1994 39% of the New Approved Drugs were of natural origin, including original natural products, semi-synthetically prepared from products and synthetic products based on natural product models(Balandrin et al., 1993; Pitcheshwar, 2014).

Furthermore, in order to prolong the storage stability of foods and to reduce the damage to human body, synthetic antioxidants are used for industrial processing in addition to natural antioxidants. But according to toxicologists and nutritionists, the side effects of some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have already been documented. For example, these substances can show carcinogenic effects in living organisms. From this point of view, governmental authorities and consumers are concerned about the safety of the food and the consequences on health. Therefore, there is a growing interest into the investigation of potentially active natural phytochemicals over using synthetic ones.

The harmful effect of free radicals causing potential biological damages are termed as oxidative stress and nitrosative stress (Ridnour et al., 2005). In order to counteract intracellular damage by free radicals; cells have developed a so-called *intracellular antioxidant system*. This process transforms free electrons into a nonreactive form by proteins (enzymes). Antioxidants regulate oxidative reactions by inhibiting, delaying or hampering the oxidation of the substances (Balandrin et al., 1993).

Plant *Verbena officinalis* Linn. (Family: *Verbenaceae*) commonly known as Verveine and found in moderate climatic region. *Verbenaceae* is represented by 31 genera and 45 species in Ethiopia. *Verbena officinalis* L. grows in all temperature regions of the globe and is a weed that is found in semi-arid environment of the world(Rehecho et al., 2008). It is a very easily grown plant; it succeeds in any moderately fertile well drained but moisture retentive soil in a sunny position. Plants are very tolerant of neglect and will maintain themselves for a number of years even growing in dense weed competition(Rehecho et al., 2008). *Verbena Officinalis* (Fam.*Verbenaceae*) is a perennial herb with several stiffly erect stems, which can be found in West Asia, North Africa and throughout Europe. It is known for its antidepressant and anticonvulsant effect as well as its use for the treatment of jaundice, cough, cold and digestive problems. Its use for healing liver and gallbladder disease and nervous exhaustion has also been recorded.

It is useful when taken internally in the treatment of ailments such as headaches, fevers, nervous exhaustion, depression, and gallbladder problems and insufficient lactation (Kou et al., 2013). Externally, it is used to treat minor injuries, sores neuralgia and gum disease. The plant is harvested as flowering begins in the summer and dried for later use. Some remarkable results have been obtained when the plant is used in the treatment of certain tumors. The root is astringent; it is used in the treatment of dysentery (Rehecho et al., 2008). In some part of Ethiopia, it is used traditionally to treat dysentery, impotency and counter acting effect of snake poison.

MATERIAL AND METHODS

Sample collection procedure

The plant material was collected from Bahir Dar city. The roots and leaves were cut in to pieces, washed with tap. The roots and leaves were placed in shade region open to air at room temperature for ten days. The dried roots and leaves were grounded into powder.

Extraction procedure

The powdered root and leaves of *V. officinalis L* were extracted with four different solvents (80% methanol, 90% methanol, 100% methanol and chloroform). A 100 mL solvent was added to 10g of dry powder root and leaves of *Verbena officinalis* at room temperature. The mixtures were extracted in electrical shaker for 48h and then the mixtures were filtered and concentrated at 35 ^{0}C.

Table 1: The result of phytochemical identified from different root extracts of *V. officinalis*

phytochemicals	Solvent				Color observed
	90% methanol	100% methanol	80% methanol	chloroform	
Glycosides	++	+	+	_	Brown ring
Saponinis	++	++	_	_	White foam
Flavanoids	++	+	+	+	Intense yellow
Terpenoids	++	++	+	+	Reddish brown precipitate
Tannins	+	+	+	_	Dark green
Polyphenols	++	+	+	+	Green-blue

Table 2: The absorbance of quercitin at different concentration at 510 nm

Concentration (ppm)	Absorbance
2	0.080±0.0023[b]
4	0.110±0.0031[c]
6	0.154±0.0012[a]
8	0.191+0.0020[b]

Table 3: Total flavonoid content (TFC) of different solvent extracts of *V. officinalis* root and leaves

Sample	mgQE/g 100 of dry wt
VOR_1	73.32±0.002[a]
VOR_2	86.38±0.052[f]
VOR_3	65.79±0.012[c]
VOR_4	42.39±0.032[d]
VOL_1	72.65±0.002[a]
VOL_2	68.53±0.005[b]

Table 4: Absorbance of AA at various concentrations at 700 nm

Concentration (mg/ml)	Absorbance
0.2	0.209±0.002[a]
0.4	0.347±0.003[b]
0.6	0.534 ±0.012[d]
0.8	0.661 ±0.004[c]

Table 5: Absorbance of FRAP assay for different root extracts of *V. officinalis* at different concentration measured at 700 nm in different solvents

Sample	90% methanol	100% methanol	80% methanol	Chloroform
10%	0.340 ±0.001[a]	0.337 ±0.001[a]	0.301±0.001[a]	0.221±0.001[a]
20%	0.4978±0.002[b]	0.472 ±0.002[b]	0.352±0.002[b]	0.287±0.001[a]
30%	0.610±0.002[b]	0.532 ±0.021[c]	0.425±0.002[b]	0.302±0.003[b]
40%	0.661±0.003[c]	0.612 ±0.033[d]	0.502±0.003[c]	0.330±0.004[c]

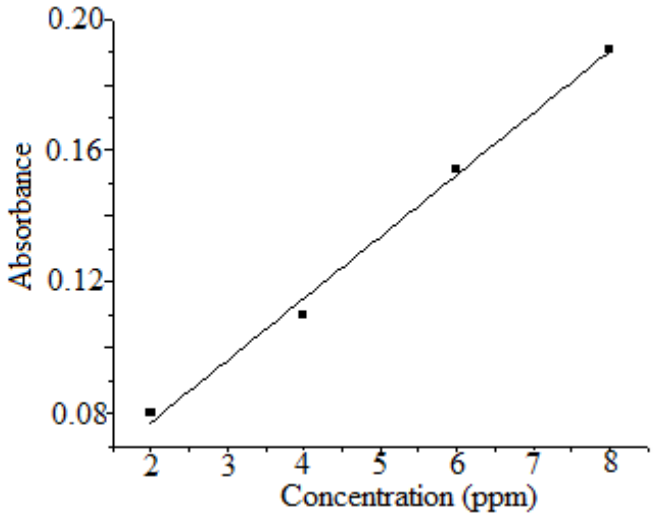

Fig. 1: Calibration curve of quercition at different concentration

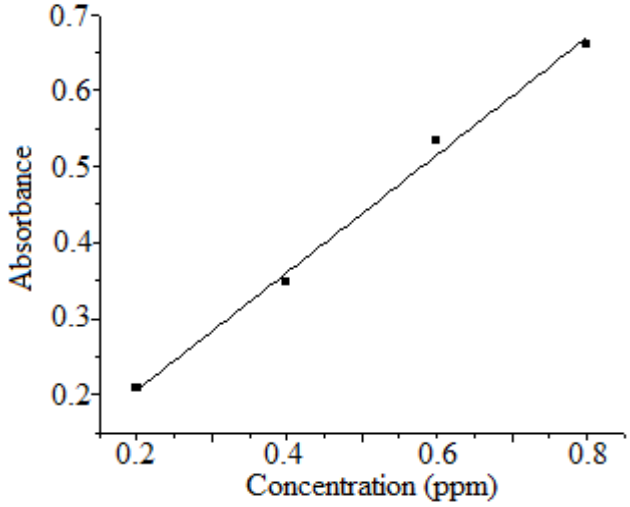

Fig. 2: Calibration curve that shows the absorbance of ascorbic acid at different concentration at 700 nm.

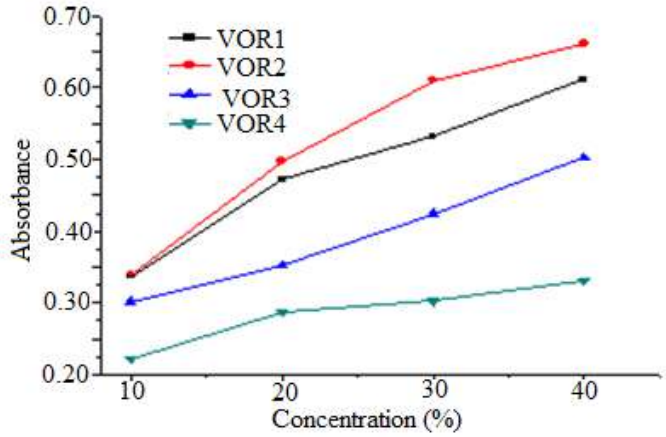

Fig. 3: Reducing power of *V. officinalis* root extracts, expressed as absorbance at 700 nm

Finally, the crude extracts were labeled as VOR_1,VOR_2, VOR_3 and VOR_4 for *V. officinalis root* extracts using 90%, 100%, 80% methanol and chloroform solvents respectively while VOL_1 and VOL_2 represent extracts of *V. officinalis* leaves using 90% aqueous methanol and 100% methanol respectively.

Determination of antioxidant activities

Reducing power assay: The reducing power of the prepared extracts was determined according to the method of Oyaizu with some modification (Oyaizu et al. 1986). Different concentrations (10%, 20%, 30%, 40%, (v/v)) of *Verbena officinalis* root and leaf extracts were prepared using different solvents (90% methanol, 80% methanol, 100% methanol and chloroform). From each sample, 2.5mLextracts were taken and mixed with 2.5mL of 200mM sodium phosphate buffer (pH = 6.6) and 2.5mL of potassium ferricyanide solution(1%). The mixtures were incubated at 50°c for 20min. Then, 2.5mL of trichloroacetic acid (TCA) solution (10%w/v) was added and the resulting mixture was centrifuged at 3000rpm for 10min. The upper layer was filtered (2.5mL) and mixed with 2.5mL of distilled water and 0.5mL ferric chloride solution (0.1% w/v). Finally, absorbance was measured at 700 nm.

DPPH radical scavenging activity determination: Based on the method described by Brand Manzorro, the antioxidant activity of *V. officinalis* root and leaves were assessed on the basis of free radical scavenging effect of 2,2 -diphenyl-1-picrylhydrazyl (DPPH)(Louli et al., 2004). 90% aqueous methanol, 100% methanol and chloroform extracts with different concentrations (40%,30%, 20%, 10%(v/v)) of *V. offiinalis* root and leaves were prepared to determine DPPH radical scavenge values. 5mL of 0.004% of DPPH solution was mixed with 1mL of each crude extract. The solutions were kept in dark for 30min and absorbance of the mixture was measured at 517nm using Uv-vis spectrophotometer.

Determination of antimicrobial activity

The disk diffusion method with Muller Hinton agar was used to evaluate antimicrobial activity. Muller Hinton Agar was melted and then cooled and finally poured into sterile Petri dishes to get a solid plate. Then, one day old fresh culture of bacteria was used for inoculums preparation. A suspension was kept till it reached the turbidity equal to 0.5 MacFarland standard solutions which is equivalent to 1.5×10 CFU/ml (Andersen, 2004; Valko et al., 2006). Using a sterile cotton swab, bacterial cultures were swabbed on the surface of sterile agar plates. The dried plant extracts were re-suspended to 20mg/mL in dimethyl sulfoxide and sonicated to dissolve and sterilize the extracts. Sterilized 5mm discs was impregnated with 50µl of extract and placed on the surface of agar plates inoculated with a microbial culture. Each extract was tested in triplicate. Streptomycin sulphate (40mg/disc) was served as a control. The plates were incubated at 37°C for 24h. The diameter of the inhibition zones was measured in millimeter. Three replicates were kept in each case and average values were taken.

Table 6: FRAP assay absorbance measurement for different leaf extracts of *V. officinalis* with different concentration at 700 nm

Sample	90% methanol	100% methanol
10%	0.330±0.003[c]	0.280±0.003[c]
20%	0.431±0.001[a]	0.321±0.001[a]
30%	0.580±0.002[b]	0.456±0.023[d]
40%	0.630±0.012[d]	0.630±0.002[b]

Table 7: Ferric reducing antioxidant power of the *v. officinalis root and* leaf extracts with different solvent (mgAAE/100g)

Sample	mgAAE/100g ext
VOR_1	372.93±0.004[b]
VOR_2	255.58±0.021[c]
VOR_3	196.67±0.003[a]
VOR_4	129.41±0.026[d]
VOL_1	281.98±0.003[a]
VOL_2	213.78±0.004[b]

Table 8: Absorbance of AA at various concentrations at 517 nm

Concentration	Absorbance	%in habitation
25ppm	0.253±0.000[a]	76.53±0.002[c]
50ppm	0.172±0.000[c]	84.31±0.001[a]
75ppm	0.780±0.001[d]	88.51±0.001[b]
100ppm	0.031±0.001[b]	95.15±0.002[c]

Table 9: Absorbance of different root extracts against DPPH solution at different concentration

Concentration	90% methanol	100% methanol	80% methanol	Chloroform
10%	0.271±0.002[b]	0.287±0.001[a]	0.305±0.001[a]	0.317±0.023[b]
20%	0.213±0.002[b]	0.227±0.011[c]	0.213±0.011[c]	0.305±0.001[a]
30%	0.123±0.003[c]	0.148±0.002[b]	0.185±0.015[d]	0.284±0.031[d]
40%	0.050±0.001[a]	0.081±0.013[d]	0.102±0.003[b]	0.279±0.002[b]

Table 10: DPPH radical scavenging activity (% inhabitation) of *V. officinalis* root extracts at different concentration

Concentration	90% methanol	100%methanol	80% methanol	Chloroform
10%	43.37±0.025[c]	42.15±0.003[b]	40.05±0.002[a]	35.44±0.013[c]
20%	51.25±0.004[b]	49.29±0.013[c]	45.35±0.014[c]	39.35±0.003[b]
30%	68.36±0.023c	60.34±0.021[d]	54.36±0.021[d]	43.35±0.002[a]
40%	87.39±0.001[a]	72.25±0.001[a]	62.38±0.004[b]	45.57±0.021[d]

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) value of extracts was determined by serial dilution method .The extracts was diluted to make different concentrations. Diluted solutions of each extract were individually placed in tubes labeled 1 to 6. Tube 1 was filled with 2 ml of Muller Hinton broth including extract. 1ml of extract from tube 1 was transferred to tube 2 and diluted with Muller Hinton broth. This procedure was repeated from tube 2 to 5 and each tube was filled with 1ml Muller Hinton broth including

bacterial suspension. The resulting mixtures were incubated at 37±0.1°C for 24h. Turbidity was taken as an indication of growth and the lowest concentration which remained clear was recorded as the relative minimum inhibitory concentration. This test was done in triplicates.

Methods of data analysis

In this study, the antioxidant activity, total flavonoid content *of V.officnalis* root and leaf extracts were calculated and reported in terms of ascorbic acid (AA), and querectin (QT) equivalent per gram of extraction. The equation stated below is used for calculation.

$$W\left(\frac{mg}{g\ of\ DW}\right) = \frac{[x(PPM)(dilution\ factor)(volume\ of\ extract(ml))}{weight\ of\ dry\ sample\ in\ gram} \ldots(1)$$

Where: W = AAE, GE, QE , Dilution factor $= \frac{final\ volume}{aliquet\ volume}$

x = y-c/A

Where, y = absorbance of the sample,

C = y-intercept from calibration curve,

A= slope from calibration curve

The percentage of DPPH radical scavenging activities of *V. officinalis* root and leaf extracts were calculated using the following equation:

$$\text{DPPH radical scavenging (\%) activity} = \left[\frac{(Ao-A1)}{Ao}\right] \times 100 \ldots\ldots\ldots\ldots\ldots(2)$$

Where: A_0 = absorbance of the blank,

A_1 = absorbance of the sample.

Statistical analysis

All measurements were carried out in triplicate (n=3), and values expressed are the mean of three repetition ± standard deviation (SD). Results were subject to analysis of variance (ANVOA). The graphs as well as linear regression coefficients (R^2) were done using origin 8 software. The difference between mean were determined by the least significant difference test, and significance was defined as a confidence limit of $P < 0.05$. Letters in the form of superscript within columns showed significant difference.

RESULTS AND DISCUSSION

Phytochemical screening

The traditional medicine involves the use of different plant extracts or the bioactive constituents. This type of study provides the health application at affordable cost. Secondary metabolites are responsible for medicinal activity of plants. The quantity and quality of phytochemicals present in different parts of a plant may vary from one part to another or from plant to another plant (Kou et al., 2013). In this study, the active components of root *extracts of V.officinalis,* one of the most widely used medicinal plants, were qualitatively analyzed. Phytochemical analysis of this plant revealed the presence of various groups of phytochemicals like glycosides, saponins, phenolic compounds, tannins, flavanoids and terpenoids. The results are summarized in Table 1. Among these phytochemicals, flavanoids, terpenoids and polyphenols were detected in all root extracts. Alkaloids were not observed at all extracts but tannins are absent only in chloroform extracts (Verma and siddiqui, 2011). The results suggest that the phytochemical properties of the plant can be used for curing various ailments and possess potential antioxidant.

Total flavonoid content determination

Calibration curve was constructed to determine the flavonoid content of *V. officnalis root and leaf* extracts. 2, 4, 6 and 8ppm concentration of quercitin were used to construct the calibration curve. The calibration curve was constructed by drawing absorbance verses concentration and a straight line with an equation of y = 0.01881 + 0.0397x and a linear regression coefficient (R^2) of 0.9926 were obtained.

The absorbance of the solution was taken from each sample and then the total flavonoid content of the samples in terms of quercetin equivalent mgQE per 100g of dry weight of sample was calculated using equation 1 and the result were given in Table 3.

The flavonoid content was higher in 90% methanol extract (VOR_1) (73.32 ±0.002) and the least total flavonoid content was recorded with solvent having lesser polarity (VOR_4) (42.39±0.012). The present study agrees with the result reported by Muhammad and his co-workers. According to the report the highest amount of TFC was from methanol extract and the least amount was recorded in *n*-hexane extract of *M. buxifolia* fruits.

The total flavonoid contents of methanol leaf and root extracts of *V. officinalis* were investigated and it was found that the root exhibited more flavonoid content than the leaves. Hence, the presence of flavonoids in the methanol root and leaf extracts of *V. officinalis* confirm the the traditional use of the plant for folkloric remedies.

Since it contains high proportion of polyphenols and flavonoids, it is reliable to possess antioxidant and anticancer activity. This increased level of total phenolics might be due to the presence of phenolic compounds like coumarins, flavonoids, lignans, neolignans, lignins, phenylpropenes in the *V. officinalis* plant.

Antioxidant potential determination of root and leaf extracts of *V. officinalis*

In the present study, different solvent fractions of leaf and root extracts of *V. officinalis* prepared by maceration were compared for their antioxidant efficacy, antimicrobial activity, phenolic and flavonoid contents. The extraction solvent of the plant material plays an important role in the activity of phytochemicals. Also, the assessment of antioxidant capacity of phytochemicals cannot be executed precisely by any single method due to complex nature of phytochemicals as multiple reaction characteristics and mechanisms can be involved.

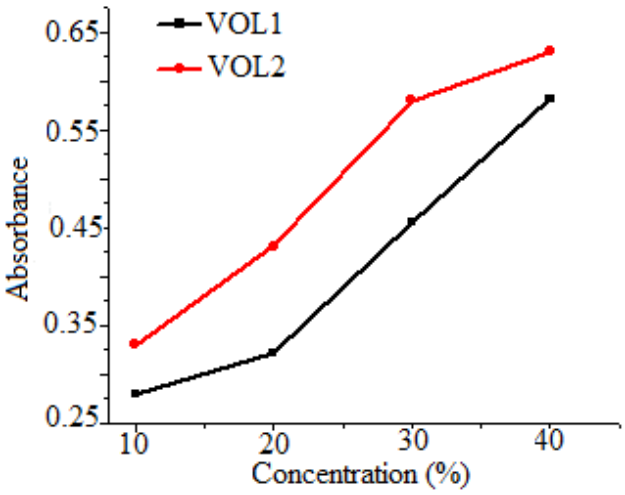

Fig. 4: Reducing power of *v. officinalis* leaf extracts expressed as absorbance at 700 nm.

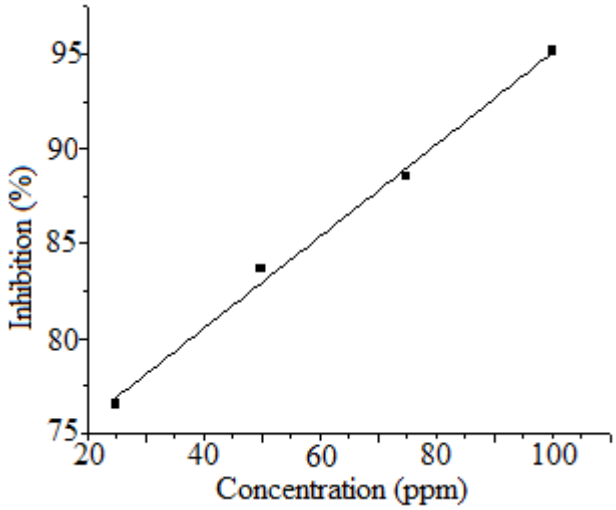

Fig. 5: Calibration curve that shows the percentage inhibition of DPPH at different Concentration.

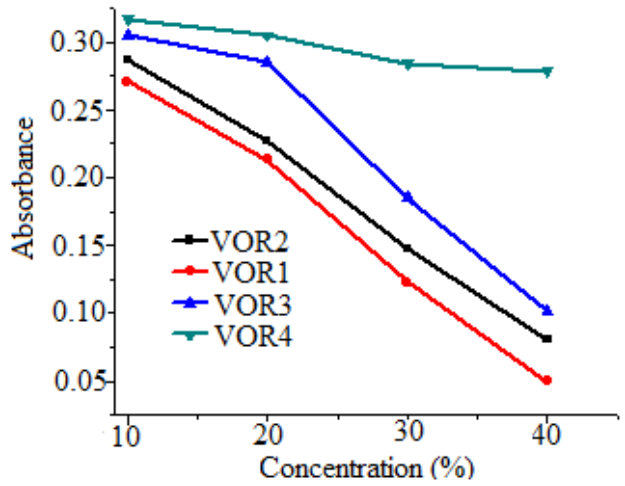

Fig. 6: Absorbance of DPPH after addition of different concentrations of *V. officinals* root extracts

So, no single assay could accurately reflect comparison in a mixed or complex system. Therefore in the present study the comparison of extracts was done by using most widely used assays viz. DPPH, FRAP, and lipid peroxidation assay. Furthermore, the UV-Vis spectrum of both extracts and the correlation between total phenolic and/or flavonoid contents was examined in order to give a direction to the search of phytochemicals responsible for their activity (Litescu et al., 2010).

Ferric reducing antioxidant power, FRAP

The reducing power of 80%, 90%, 100% methanol and chloroform root and leaf extracts of *V. officinalis* were determined using Oyaizu method (Arulpriya et al., 2010). Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric/ferrous complex that has an absorption maximum at 700 nm (Valko et al., 2006).

First, the calibration curve was prepared to determine the antioxidant activity of *V. offcinalis* extracts in terms of ascorbic acid equivalent. The calibration curve was plotted as absorbance verses concentration of ascorbic acid (0.2, 0.4, 0.6, 0.8) mg/ml and the value of the absorbance obtained corresponding to concentration are given in Table 4 with linear regression coefficient (R^2) 0.99217.

In this assay the yellow color of the test solution was changed to various shades of green and blue depending on the reducing power of each extract. In this method, the presence of antioxidants causes the conversion of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, by measuring the formation of pearls Prussian blue at 700nm, the Fe^{2+} concentration can be monitored; a higher absorbance at 700nm indicates a significant reducing power of the extract. The reducing power of different extracts of *V. officinalisis* were expressed as absorbance per specific amount of extracts (10%, 20%, 30%, and 40%) (V/v) as presented in Fig. 2 and Table 5. When the *V. officinalis* sample is mixed with yellow colored $k_3[Fe(CN)_6]$ complex in the presence of phosphate buffer. The color of the solution remains the same. Prussian blue color developed following the incubation of the resulting solution at 50°c for 20min and with subsequent addition of ferric chloride .This color change shows the reduction of ferric to ferrous ion in the presence of a reducing agent (antioxidants). The water soluble antioxidants such as polyphenolic components and other substance that constitute the plant are the most responsible reducing agent in the *V. officinalisis* extracts. Based on the graph and analyzed data, all the extracts exhibited some degree of reducing power. The reducing power increases with concentration.

The increase in absorbance of the plant extracts (Fig. 3, Table 5) indicates an increase in reducing power of the tested samples. This indicates that polar compounds are more responsible for antioxidant activities which could be better extracted with high polar solvents. The weaker reductive activity of 80% methanol extract was probably due

to hydrolysis of highly polar and active molecules as the amount of water increases. Therefore the reduction power of the *V. officinalis* extracts serve as a significant indicator of its potential activity.

Out of root and leaf extracts of *V.officinalis,* methanol root extracts showed more reducing power than the leaves. When concentration increased, root extracts exhibited more reducing activity than the leaves. For instance, methanol root and leaf extracts of *V. officinalis* showed absorbance values of 0.340 and 0.330 at 10%v/v whereas it was 0.661 and 0.630 at 40%v/v respectively (Table 5 and Table 6).

Ferric reducing antioxidant power (FRAP) of the *v. officinalis root and* leaves extracts with different solvent (mgAAE/100g) of dry weight of the sample is calculated using equation 1 and the result were given in Table 7.

Determination of radical scavenging activity (DPPH)

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH. As consequence, the absorbance's decreased from the radical changes from DPPH to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (Arulpriya et al., 2010). Calibration curve was prepared to determine the antioxidant activity of *V. officinalis* root and leaf extracts in terms of ascorbic acid equivalent using DPPH radical scavenging methods. Ascorbic acid equivalent antioxidant capacity is expressed as mg of AAE per 100g of *V.officnalis* root and leaf extracts. The calibration curve was plotted as absorbance verses different concentration of ascorbic acid (25ppm, 50ppm, 75ppm and 100ppm) and the value of the absorbance obtained corresponding to concentrations are given in Table 8. The percentage inhibitions against concentration are given in Fig. 5.

The DPPH radical scavenging assay is an easy, rapid and sensitive method to screen the antioxidant activities of plant extracts**.** A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received great attention owing to its ease of use and convenience (Oyaizu, 1986). Table 9 shows the DPPH scavenging capacity of *V. officinalis* root extracts. It's percent inhibition was increased with increasing concentration. This means, as the concentration increased, the DPPH radical scavenging activity by the extract was also increased. The concentration and absorbance of the sample are inversely related. The absorbance was decreased as the concentration of the sample increased. The original purple color of DPPH was changed in to yellow (Figure 12) when the reagent was added to the extract which confirmed the reaction of the extract with the DPPH radical. This can be quantified by measuring its absorbance. The result reveals that *V. officinalis* is rich in antioxidant compounds and the effect can be expressed qualitatively with color intensity as it is indicated in Fig. 6.

The percentage radical scavenging activity of the *V. officinalis* root extracts is depicted in Fig. 7 and Table 10. The

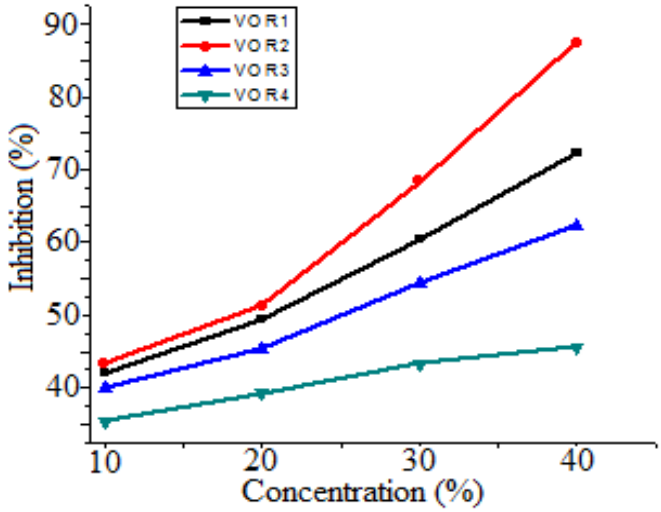

Fig. 7: DPPH radical scavenging activity of *V. officinalis* root extracts at different concentration

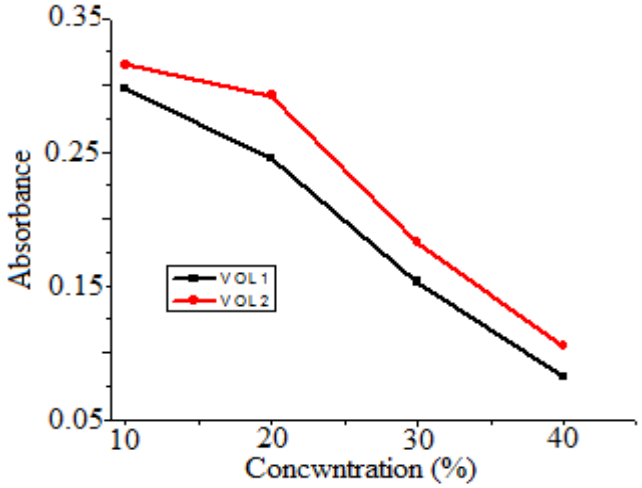

Fig. 8: DPPH radical scavenging activity of *V. officinalis* leaf extracts at different concentration

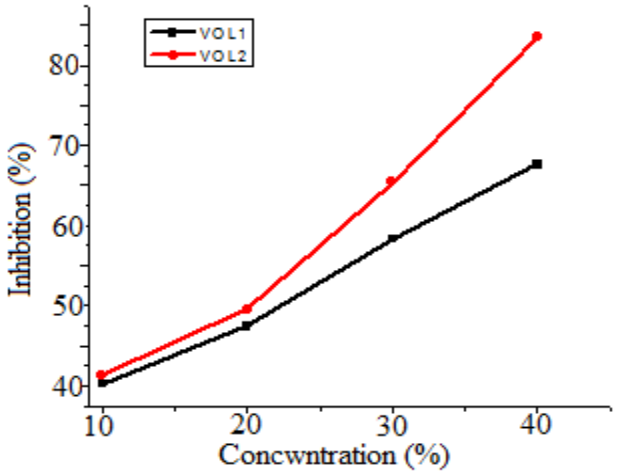

Fig. 9: % inhibition versus concentration plot of leaf extracts for DPPH scavenging activity

scavenging effect of different root extracts on the DPPH radical decrease in the order: 90% methanolic extract > 100% methanolic extract > chloroform extract. 90% methanol root extract of *V. officinalis* at 40% (v/v) concentration exhibited the highest radical scavenging activity (87.39% ±0.001) and the least (45.57% ±0.021) activity was observed when chloroform root extracts of *V. officinalis* was used at the same concentration. Highest percentage inhibition for 90% methanol extract could be due to the presence of DPPH scavenges components in higher concentration than other extracts. As the concentration of phenolic compound increase, the degree of hydroxylation of the phenolic compound also increase which results increase in scavenging activity (% inhibition) of DPPH (Louli et al., 2004). This is possible when the polarity of the solvent used to extract samples increases. That is why 90% methanol

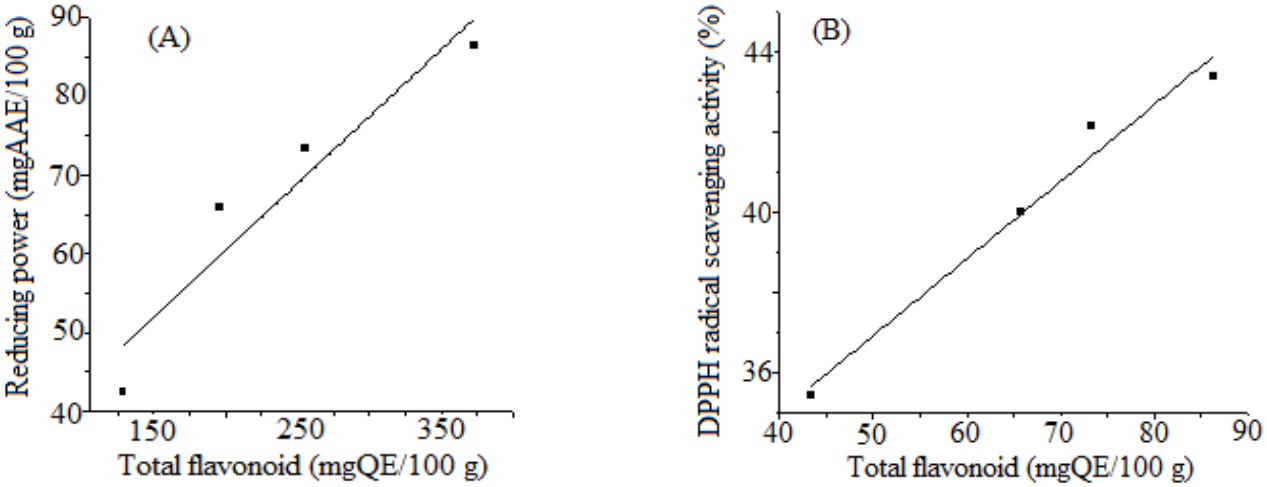

Fig 10: Correlation between reducing power, FRAP Vs TFC (A) and radical scavenging DPPH Vs TFC (B)

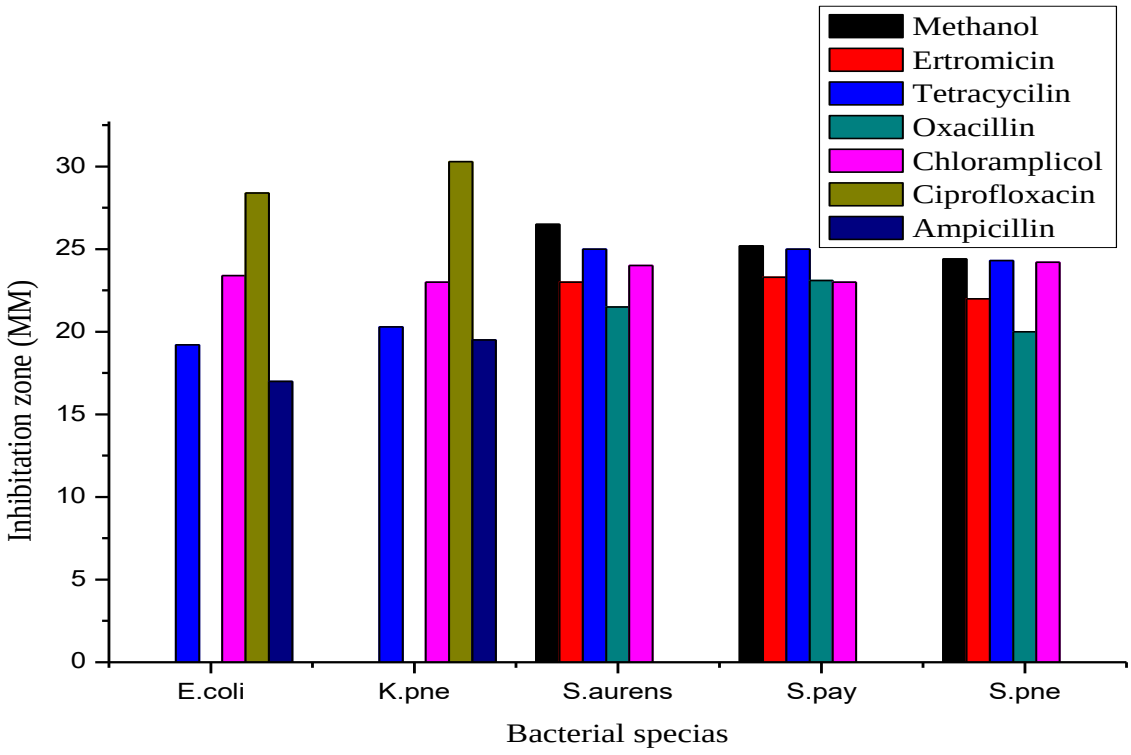

Fig. 11: Comparison of inhibition zone among crude extracts of *V. officinalis* root against standard, gram positive and gram negative bacteria

extraction of root of *V. officinalis* which has highest DPPH radical scavenging activity showed highest antioxidant activity in comparison to other extracts. The presence of polyphenol in the root extract of tested plants increases the probability of reaction with free radical which lead to decrease in the amount of free radical. The percentage inhibitions of the sample were calculated using equation 2. Qualitatively, DPPH radical scavenging activity was confirmed by color change (purple to yellow) when the extracts were added to DPPH solution. Different intensity of yellow color formation indicated the difference in DPPH radical scavenging activity between the samples. Generally, from the results, it was concluded that antioxidant activity and phenolic compounds were predominant in the 90% methanol prepared by maceration method.

In this assay, both methanol root and leaf extracts were investigated. At higher concentration, root extracts showed more scavenging activity than leaves (Table 11 and Fig. 8).

Correlation between different antioxidant assays against TFC

In this study, different antioxidant assays such as: DPPH radical scavenging capacity, total flavanoids and reducing potential were used to evaluate the antioxidant activity of *V. officinalis* leave and root extracts. Results of correlation between different antioxidant assays against TFC are given in Fig.10. TFC showed a good correlation with DPPH (R^2 = 0.962) and moderate correlation with Reducing power assay (R^2 = 0.860) in all root extracts of *V. officinalis*. The difference in correlation may be due to the diverse mechanisms followed by the assays chosen (Ugochukwu et al., 2013).

In general, the antioxidant activity demonstrated by FRPA and DPPH assay involving *V. officinalis* root and leaf extracts was excellent. In this study, correlation using radical scavenging, DPPH, method has better correlation coefficient with total flavonoid content. The probable reasons for this result might be due to better interaction of flavonoid molecules with DPPH radical than it does with FRAP. Moreover, DPPH assay has good stability, credible sensitivity, simplicity and feasibility (Bhagat et al., 2012).

The antioxidant activities of 90% methanol extract (90% MF), 80% methanol (80% MF), 100% methanol (100% MF), chloroform (CF) and petroleum ether (PF) from the *V. officinalis* root and leaves fractions were investigated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing/antioxidant power (FRAP) assays. The results showed that all the extracts possessed potent antioxidant activity. There was a significant linear correlation between the total phenolics concentration and free radical scavenging activity or the ferric reducing power of the extract and fractions. Especially, DPPH is strongly correlated with TPC than FRAP assay. Among the extract and fractions, the 90% MF fraction exhibits the best antioxidant performance.

Determination of antibacterial activity of *V. officinalis* root extracts

Agar disc diffusion method: Methanol extracts of *V. officinalis* root exhibited prominent antimicrobial activity against some bacteria species. The results of inhibition obtained by methanol extract of *V. officinalis* root and a control against each tested microorganism (gram positive and gram negative bacteria) are given in Fig. 11and Table 12, 13 and 14.

The methanol fraction showed maximum antibacterial activity against *S. aureus* (ATCC 25923) with diameter of 26.5±0.05mm at the concentrations of 128mg/mL respectively. There was no large inhibition zone difference observed among the methanol root extracts against gram positive bacteria such as *S. payogen* and *S. pneumonie* (multidrug resistance). But methanol extracts of *V. officinalis* root didn't show any activity against all gram negative bacteria (*E. coli* (multidrug resistance) and *K. pneumoniae* (multidrug resistance)).

The presence of zone of inhibition around the well was considered positive. The extracts were found to exhibit marked inhibition of bacterial growth in a dose dependent manner. Among bacteria, gram positive bacteria have shown higher sensitivity towards extracts compared to gram negative bacteria. Some inhibitions caused by methanol fractions were weaker than that of standard antibiotic but some of the root extracts showed strong activities than the controlled used.

In general the result of this study has shown that some of the isolated bacteria were susceptible to methanol extract of *V. officinalis* root of in agar well diffusion assay. Thus *V. officinalis* plant could provide effective ways to control microbial infection especially caused by those multidrug-resistance gram positive pathogens. Therefore, it is promising that the plants can be used as potential antimicrobial remedy to inhibit bacterial growth.

Minimum inhibitory concentration (MIC)

The MIC value of *V. officinalis* against tested bacteria is indicated in Fig. 19 and Table 15. The methanol fraction of the plant showed good MIC value of 4 mg/mL, 8 mg/mL and 8mg/mL against *S. aureus , S.pyogen and S.pneoumonie,* respectively. Whereas as the gram negative bacteria (*E.coli* and *K.pneoumonie*) were resistant even at 64 mg/mL.

CONCLUSIONS

Based on the present study, it can be concluded that all root and leaf extracts of *V. officinalis* exhibited different levels of antioxidant activity in all methods of antioxidant assays. The root of *V. officinalis* contains potential antimicrobial components that may be of great use for the development of pharmaceutical industries as an alternative therapy against various bacterial diseases. This study has supported the antioxidant and antibacterial activity claim of *V. officinalis* in folk medicine. The present data would certainly help to ascertain the potency of the tested medicinal plant as potential source of natural antioxidants to be used for nutraceutical and functional food applications.

Table 11: Absorbance of DPPH solution at different concentration in different leaf extracts

Concentration	Absorbance of 90% methanol	% inhabitance 90% methanol	Absorbance of 100% methanol	% inhabitation of 100% methanol
10% v/v	0.297±0.0021[b]	41.35±0.0014[b]	0.315±0.0012[b]	40.21±0.0004[a]
20% v/v	0.245±0.0014[a]	49.54±0.0023[c]	0.292±0.0002[a]	47.38±0.0033[c]
30%v/v	0.153±0.0015[a]	65.38±0.0032[d]	0.182±0.0024[c]	58.36±0.0025[b]
40%v/v	0.082±0.0033[c]	85.50±0.0004[a]	0.105±0.0031[d]	67.57±0.0031[c]

Table 12: Comparison of inhibition zone among crude extracts of *V. officinalis* root against standard drug resistance gram positive and gram negative bacteria using agar disc diffusion method

	Bacterial strains				
	Gram negative bacteria		Gram positive bacteria		
Extracts (methanol)	*E.coli*	Kp	Sp_1	Sp_2	Sa
	0.00	0.00	25.2 ± 0.03	24.4 ±0.12	26.5±0.05

Where, kp = K. pneumonie (multidrug resistance), EC1 = E.coli (ATCC25922), sp1 = S.payogen, sp2 = S. pneumonie (multi drug resistance), Sa = S.aureus (ATCC25923).

Table 13: Comparison of inhibition zone among crude extracts of *V. officinalis* root against standard using drug resistance gram positive bacteria

Tested agents	Bacterial strains		
Positive control	Sp_1	Sp_2	Sa
Ertromaicine	23.3±0.15	22±0.22	23±0.05
Tetracycline	25±0.51	24.3±0.13	25±0.63
oxacillin	21. 5±0.33	23.1±0.32	20±0.44
Chloramphenicol	24±0.15	23±0.07	24.2±0.32

Table 14: Comparison of inhibition zone among crude extracts of *V. officinalis* root against standard using drug resistance gram negative bacteria

Tested agents	Bacterial strains	
Positive control	E. *coli*	*K. pneumoniae*
Cipofloxaclin	28.4±0.04	30.3±1.23
Tetracycline	19.2±0.12	20.3±0.89
Chloramphenicol	23.4±1.03	23.0±0.35
Ampicilline	17.0±0.54	19.5±0.07

Table 15: MIC determination of *V. officinalis* root extracts against gram positive and gram negative bacteria

Test bacteria	64 mg/mL	32 mg/mL	16 mg/mL	8 mg/mL	4 mg/mL	2 mg/mL	MIC (mg/mL)
S.*aureus*	_	_	_	_	_	+	4
S.*pyogen*	_	_	_	_	+	+	8
S.*pneomonie*	_	_	_	_	+	+	8
E.*coli*	+	+	+	+	+	+	_
K.*pneomonie*	+	+	+	+	+	+	_

Kay - = inhibition of bacterial growth, + = Bacterial growth

The antioxidant capacities and total phenolic contents of *V. officinalis* medicinal plant associated with the treatment of rheumatic diseases were evaluated using the FRAP and DPPH assays as well as the Folin-Ciocalteu method, respectively. Overall, this medicinal plant had relatively high antioxidant capacities and total phenolic contents. A significant correlation between the FRAP values and DPPH values with TPC or TFC suggested that antioxidants in this plant were

capable of reducing oxidants and scavenging free radicals. A strong linear correlation between the free radical scavenging capacity and total phenolic contents indicated that the phenolic compounds could be the main contributors to the antioxidant capacities of *v. officinalis* plant. Methanol extracts of the plant showed the highest antioxidant capacities and total phenolic contents among all other tested extracts, and therefore, *V. officinalis* could be potential rich sources of natural antioxidants. Because of its strong antioxidant capacities, this plant is also potential in anti-inflammatory abilities. *V. officinalis* plant was a good source of phytochemicals and it possesses high antioxidant properties. It regular consumption will go a long way in reducing diseases related to aging. The extracts of these plants can be used as additives in our edible oils in combating the problem of lipid rancidity.

The present study indicated that *V. officinalis* roots and leaves are rich in flavonoids and exhibit strong antioxidant activity in the three methods tested (DPPH, FRAP and lipid oxidation assays). Its MeOH soluble fractions showed the strongest antioxidant activity. The antioxidant activities correlated well with their content of flavonoid compounds. Although the antioxidant activity found in an *in vitro* experiment is only indicative for the potential health benefits, these results remain significant as the first step in screening antioxidant activity of *V. officinalis* root and leaves. It can be concluded that, *V. officinalis* roots, which are consumed as folkloric remedy in Ethiopia, can be used as an accessible source of natural antioxidants with consequent health benefits.

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REFERENCES

Kovacic, P., Jacintho, J.D., 2001. Mechanisms of carcinogenesis focus on oxidative stress and electron transfer. Current Medicinal Chemistry 8(7), 773-796.

Ridnour, L.A., Isenberg, J.S., Espey, M.G., Thomas, D.D., Roberts, D.D., Wink, D.A., 2005. Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1. Proceedings of the National Academy of Sciences of the United States of America 102(37), 13147-13152.

Sies, H., 1997. Oxidative stress: oxidants and antioxidants. Experimental Physiology 82(2), 291-295.

Balandrin, M. F., Kinghorn, A. D., Farnsworth, N. R., 1993. Plant-derived natural products in drug discovery and development: an overview. In ACS symposium series (USA).

Verma, V.K., siddiqui, N.U., 2011. Bioactive chemical constituents from the plant *verbena officinalis* linn. Pharmaceutical Research 2, 39-42.

Rehecho, S., Hidalgo, O., de Cirano, M.G.I., Navarro, I., Astiasarán, I., Ansorena, D., Calvo, M. I., 2011. Chemical composition, mineral content and antioxidant activity of Verbena officinalis L. LWT-Food Science and Technology 44(4), 875-882.

Kou, W. Z., Yang, J., Yang, Q. H., Wang, Y., Wang, Z. F., Xu, S. L., Liu, J., 2013. Study on in-vivo anti-tumor activity of *Verbena officinalis* extract. African Journal of Traditional, Complementary and Alternative Medicines 10(3), 512-517.

Litescu, S. C., Eremia, S., Radu, G. L., 2010. Methods for the determination of antioxidant capacity in food and raw materials. In Bio-Farms for Nutraceuticals, Springer US. pp. 241-249.

Scheibmeir, H. D., Christensen, K., Whitaker, S. H., Jegaethesan, J., Clancy, R., Pierce, J. D., 2005. A review of free radicals and antioxidants for critical care nurses. Intensive and Critical Care Nursing 21(1), 24-28.

Andersen, J. K. 2004. Oxidative stress in neurodegeneration: cause or consequence. Nature Reviews Neuroscience 5, S18–S25.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. M., Mazur, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions 160(1), 1-40.

Jasuja, N. D., Sharma, S. K., Saxena, R., Choudhary, J., Sharma, R., Joshi, S.C., 2013. Antibacterial, antioxidant and phytochemical investigation of Thuja orientalis leaves. Journal of Medicinal Plants Research *7*, 1886-93.

Dai, J., Mumper, R.J., 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15(10), 7313-7352.

Lafay, S., Gil-Izquierdo, A., 2008. Bioavailability of phenolic acids. Phytochemistry Reviews 7(2), 301-311.

Oyaizu, M., 1986. Studies on products of browning reaction--antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 44, 307-315.

Louli, V., Ragoussis, N., Magoulas, K., 2004. Recovery of phenolic antioxidants from wine industry by-products. Bioresource Technology 92(2), 201-208.

Arulpriya, P., Lalitha, P., Hemalatha, S., 2010. Invitro antioxidant testing of the extracts of Samanea saman (Jacq.) Merr. Der Chemica Sinica 1(2), 73-79.

Kumar, P. S., Jayanthi, B., Abdul, K., Prasad, U. V., Kumar, Y. N., Sarma, P.V.G.K., 2012. Effect of Different Types of Fermentation (Inoculated and Natural Fermentation) On

the Functional Properties of Apple Tea Wine. Research Journal of Pharmaceutical, Biological and Chemical Sciences 3(2), 752-761.

Ugochukwu, S. C., Uche, A., Ifeanyi, O., 2013. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. Asian Journal of Plant Science and Research 3(3), 10-13.

Pitcheshwar, P., 2014. Phytochemical screening, anti-inflammatory activity and anti-oxidant activity assessment of black tea, green tea and rooibos tea. Journal of Biological and Information Sciences 3(4), 23-26.