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Preliminary Studies on the Antibacterial and Antioxidative Potentials of Hydroalcoholic Extract from the Whole Parts of *Artemisia vulgaris* L.

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ABSTRACT

The present study assessed the antibacterial, cytotoxicity and antioxidative capacity of hydroalcoholic extract from the whole parts of *Artemisia vulgaris* (AV) using various *in vitro* assay models. Lipid Peroxidation (LPO), Protein Glycation (PG), Xanthine Oxidases (XO) and the stable 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging assays were used to evaluate the antioxidative capacity of AV extract. The Total Phenolic Content (TPC) was estimated based on Folic Ciocalteau reagent whilst, the phenolic compounds were characterized by HPLC-DAD. Here, the cytotoxicity nature of AV extract was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent on HepG2 hepatocarcinoma cells. Furthermore, the antibacterial potential was determined by using agar dilution method against our panel of bacterial strains. A mild antibacterial activity was exhibited against *P. vulgaris* ATCC 6830, *E. faecalis* ATCC 29212, *S. mercescens* ATCC 9986, *S. aureus* OK1 and OK3 having MIC value of 0.4 mg mL⁻¹ but less active towards other test bacteria. Similarly, the AV extract demonstrated a moderate inhibition of DPPH* (IC₅₀: 0.976 µg mL⁻¹), LPO (IC₅₀: 360 µg mL⁻¹), XO (IC₅₀: 112.51 µg mL⁻¹) and PG (IC₅₀: 968.4 µg mL⁻¹). The TPC was found to be 28.04±0.05 mg GAE g⁻¹, which corresponds to the HPLC fingerprint. The AV extract exhibited non-toxic effect towards mammalian cells having an IC₅₀ value of 246.32±5.21 µg mL⁻¹. The data showed that *Artemisia vulgaris* had a mild antibacterial and antioxidative capacity despite its low phenolic composition and at least in part support its medicinal uses in folklore medicinal system.

Key words: *Artemisia vulgaris*, antioxidant, cytotoxicity, phenolic compound, free radical, antibacterial

INTRODUCTION

The increasingly growing interest in the pharmaceutical and food industries for the substitution of antimicrobial additives and synthetic food antioxidants has necessitated the search for identifying new plant-derived products with promising therapeutic potentials (Delgado-Adamez *et al.*, 2012). Similarly, the constant exposure of man to environmental pollution and cosmic radiation have been implicated in most studies as a major cause of cardiovascular and neurodegenerative diseases in man due to imbalance between the Reactive Oxygen Species (ROS) and antioxidant

defense mechanism (Oyedemi *et al.*, 2010). To overcome this challenge, natural antioxidants such as polyphenols, thiols, ascorbic acid, β-carotene and tocopherol especially from plant origin has been suggested as potential molecules capable of resisting oxidative damages to human cells without or little deleterious effect compared with synthetic antioxidants (Akira *et al.*, 2014). On the other hands, the risk of antibiotic resistance emanated from indiscriminate use of antimicrobial drugs for the treatment of infectious human diseases has increased and now become a global concern (Sibanda and Okoh, 2007). Recent studies confirmed that majority of the world population are engaged in the use of herbal remedies,

preferring them to Western medicines (WHO., 1991). This could be associated to the myriad of secondary metabolites embedded in medicinal plants with proven therapeutic potentials which are now applied in clinical practice (Oyedemi *et al.*, 2012). These natural products have therefore, been proposed as a potential source of novel molecules for the treatment of human ailments, using them may be more cost-effective and engender fewer side effects compared with Western medicine. However, only a small number of these botanicals has been evaluated and received rigorous scientific evaluation to assess their efficacy.

Artemisia vulgaris L (AV: mugwort) is naturalized in the Eastern Cape province, South Africa but indigenous to Europe, Iran, Siberia and North Africa (Huxley, 1992). It is a tall aromatic perennial herb, belongs to the family Asteraceae (Wright, 2002). The infusion of the leaves and stem of AV is used in folk medicine for the treatment of wide range of health related problems such as diabetes, epilepsy, depression, insomnia, malaria, tumor, dermatitis, bacterial infection, menopausal and menstrual complaints (Lewis and Elvin-Lewis, 2003; Avila and Fetrow, 2004; Gruenwald, 2004). Previous studies have shown that AV is a potent antihypertensive (Tigno *et al.*, 2000) antioxidant in raw sheep meat (Luo *et al.*, 2007), anti-inflammatory (Tigno and Gumila, 2000) and hepatoprotective agent (Gilani *et al.*, 2005). The essential oils components: α -thujone, caryophyllene oxide and 1,8 cineole extracted from the aerial part of this botanical as major components (Erel *et al.*, 2012) are identified with robust insect repellent and fumigant activity (Wang *et al.*, 2006). So far, the main chemical components isolated from the leaf extracts of *Artemisia vulgaris* includes artemisic acid and artemisinin B reported to have a strong antitumor activity (Sun *et al.*, 1992). To the best of our knowledge, there is scanty or no scientific information on the antibacterial properties of *Artemisia vulgaris* L against our panel of bacterial strains as well as the antioxidative properties of the hydroalcoholic extract from the whole plants grown in South Africa.

The present study, therefore aimed at investigating the antibacterial, cytotoxicity, phenolic composition and antioxidative capacities of the hydroalcoholic extract from the whole *Artemisia vulgaris* used in South Africa traditional medicine.

MATERIAL AND METHODS

Plant collection and extract preparation: The whole plant of *Artemisia vulgaris* L was purchased in September 2011 from an herbalist with indigenous knowledge of plant use in South Africa and later authenticated by Prof. R.M. Coopoosamy (Department of Nature Conservation and Ethnobotany, Mangosuthu University of Technology). A voucher specimen (SOAV1) was prepared and deposited at the above mentioned University herbarium. The whole plant was air dried at room temperature for 14 days. It was grinded to powder form and stored in an airtight container for future use. Fifty gram of the dried powdered plant material was extracted in 70% ethanol on a mechanical shaker (Labotec Scientific Orbital Shaker, SA) for 48 h, the extract was filtered using a Buchner funnel and

Whatman No.1 filter paper. The volume was concentrated under vacuum at 40°C to recover ethanol and finally freeze dried, yielding 8.64 g of dark extract characterized with choking smell. The 70% ethanol was chosen as a solvent for extraction in accordance to the folklore preparation.

Bacterial strains: Bacteria isolates used in this study are reference strains obtained from the Applied and Environmental Microbiology Research, Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. These bacteria were chosen for investigation based on their pathological effects on humans and food deterioration. Four gram-positive bacteria: *Bacillus cereus* (10702), *Bacillus pumilus* (ATCC 14884), *Enterobacter faecalis* (KZN) and seven gram-negative bacteria: *Proteus vulgaris* (KZN), *Serratia mercersens* ATCC (9986), *Acinetobacter calcoaceticus* (UP), *Pseudomonas aeruginosa* (ATCC 19582), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (KZN). *Staphylococcus aureus* OK1 and *Staphylococcus aureus* OK3 isolated from marines were included for susceptibility test. The bacterial strains and isolates were grown at 37°C overnight and maintained on nutrient agar, the inoculum was then standardized following the McFarland turbidity of 0.5 at 600 nm to achieve 5×10^5 CFU mL⁻¹.

Cells culture and maintenance: The HepG2 cell lines (American Type Culture Collection) were purchased from American Type Culture Collection (Manassas, VA). The HepG2 cells were replenished with new growth medium every 2-3 days and sub-cultured every 3-4 days when they are 80% confluent. Cells were maintained in Dulbecco Modified Eagle's Medium (DMEM) (Invitrogen, UK) containing 10% foetal bovine serum (FBS; GIBCO 10010), antibiotics {Penicillin (100 IU mL⁻¹) and streptomycin (100 μ g mL⁻¹)} incubated at 37°C in a 5% CO₂ humidified atmosphere and split when confluent. Cells were allowed to grow in the media to 60-80% confluence before harvesting for the MTT cytotoxicity assay. The cell density was adjusted to 7.5×10^4 cells mL⁻¹ before exposed to different concentration of plant extracts prepared in dimethyl sulfoxide (DMSO) with a final concentration of 1% v/v.

Total Phenolic Content (TPC): Total phenolic content present in the hydroalcoholic extract from *Artemisia vulgaris* was determined for the first time using Folin Ciocalteu reagent (FCR) as described by Lister and Wilson (1991) with slight modification. 0.5 mL of AV extract solution (1 mg mL⁻¹) was added to the reaction mixture of 2.5 mL of 10% FCR and 2 mL of Na₂CO₃ (2% w/v). The resulting mixture was incubated at 45°C with shaking for 15 min and the absorbance was recorded at 765 nm. The TPC was extrapolated from the standard curve expressed as Gallic Acid Equivalents (GAE) using the formula: $T = C.V/M$. Where T is the TPC (mg g⁻¹) in the extract, C is the concentration of gallic acid established from the calibration curve, V is the volume of the extract, mL and M is the weight of the extract (g).

Free Radical Scavenging Activity (FRSA) using DPPH

assay: The antioxidative capacity of *Artemisia vulgaris* was determined using a modified method of DPPH assay described by Yan *et al.* (1998). Twenty five milligram of the crude extract ($3.125\text{-}0.1\text{ mg mL}^{-1}$) was dissolved in 5 mL of methanol, 1.5 mL of this solution was added to 1.5 mL of 0.1 mM DPPH prepared in DMSO. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was read at 517 nm after 2 min. The FRSA was calculated as $[1-(A_i-A_j)/A_c] \times 100$, where A_i is the absorbance of 1.5 mL of the crude extract solution mixed with an equal volume of the DPPH solution, A_j is the absorbance of 1.5 mL of the crude extract solution mixed with an equal volume of DMSO while, A_c is the absorbance of a blank (1.5 mL of the DPPH with an equal volume of methanol). Caffeic acid was used as reference drug.

Protein glycation (PG) inhibition: Inhibition of albumin glycation by *Artemisia vulgaris* extract was done in accordance to the method described by Matsuura *et al.* (2002). Six tubes were prepared for the blank and each of the extract concentrations ($0.25\text{-}5\text{ mg mL}^{-1}$) was mixed with 400 μL of 1 mg mL^{-1} BSA and 90 μL of 1.11 M glucose in 50 mM phosphate buffer (pH 7.4). Three of these samples were incubated in a 60°C water bath for 24 h with 10 μL of extracts or distilled water while another three tubes were kept at 4°C. After incubation, 100 μL of each sample was transferred to a new tube and 10 μL of cold 100% TCA was added to each tube. They were vortexed and centrifuged at $15000 \times g$ for 4 min at 4°C. The pellet [containing Advanced Glycation End products (AGE)] was collected and dissolved in 400 μL of alkaline Phosphate Buffered Saline (PBS). The fluorescence intensity was measured using a Multiscan MS microtitre plate reader with an excitation/emission wavelength of 370/440 nm.

Lipid oxidation (LPO) inhibition assay: The inhibitory potential of hydroalcoholic extract from *Artemisia vulgaris* against lipid peroxide formation was determined using a modified thiobarbituric acid-reactive species (TBARS) assay (Dharmananda, 2003). Egg homogenate (0.5 mL, 10% in distilled water, v/v) and 0.1 mL of the methanol leaves extracts from AV was mixed in a test tube and made up to 1 mL with distilled water. A volume of 0.05 mL FeSO_4 (0.07 M) was added to the above mixture and further incubated for 30 min, to induce lipid oxidation. Thereafter, 1.5 mL of 20% acetic acid (pH 3.5), 1.5 mL of 0.8% w/v thiobarbituric acid (TBA) prepared in the reaction mixture containing 1.1% w/v Sodium Dodecyl Sulphate (SDS) and 0.5 mL of 20% w/v Trichloroacetic acid (TCA) were added, vortexed and then heated in a boiling water bath for 60 min. To avoid non-MDA (Malondiadehyde) interference due to high anthocyanin formation, another set of samples were prepared in the same manner, incubated without TBA. After cooling, 5.0 mL of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the upper layer solution was measured at 532 nm. For the blank, 0.1 mL of distilled water was used instead of the extract. The percentage inhibition (% LOI) of peroxide formation by the plant extract was calculated using the equation:

$$(1-E/C) \times 100$$

where, C is the absorbance value of the fully oxidized control and E is the change in absorbance of TBA present/absent.

Xanthine Oxidase (XO) inhibition assay: The Xanthine Oxidase (XO) inhibitory capacity of *Artemisia vulgaris* using xanthine as the substrate was measured according to the procedures of Pieroni *et al.* (2002). The plant extract solution was prepared dissolving 15 mg of each freeze-dried extract in 1.2 mL of ethanol and 1.8 mL of phosphate buffer (42 mM KH_2PO_4 and 15.6 mM $\text{Na}_2\text{B}_4\text{O}_7$, pH 7.4). The resulting solutions were centrifuged and 0.2 mL of the supernatant was added to the assay mixtures (2.7 mL of phosphate buffer, 2.0 mL of 0.15 mM xanthine prepared in 0.05 M NaOH). A volume of 0.1 mL XO (0.39 U/mL dissolved in phosphate buffer) was added in order to start the reaction as described by Noro *et al.* (1983). The assay mixture was incubated at 37°C for 60 min, followed by measuring uric acid production at 295 nm. The absorbance was measured against a blank solution prepared in the same manner with 0.2 mL of ethanol instead of the sample supernatant. The XOI was expressed as the percentage inhibition of XO formation, calculated as $(1-B/A) \times 100$, where A is the change in absorbance of the assay without the plant extract and B is the change in absorbance of the assay with the plant extract using caffeic acid as positive control (absorbance with XO-absorbance without XO).

Determination of Minimum Inhibitory Concentration

(MIC): The Minimum Inhibitory Concentrations (MICs) of the antibiotic and AV extract were determined using the standard method described by Clinical Laboratory Standards Institute (2004). The stock antibiotic concentrations diluted in the nutrient broth (Biolab) were incorporated into the molten agar at 50°C to give a working concentration between 0.001 and 0.824 mg mL^{-1} and extract between 50-0.05 mg mL^{-1} . A volume of 1 mL from each dilution of the extract was mixed with 19 mL of molten sensitivity test agar (Biolab) at 50°C and poured into sterile petri dishes allowing the agar to set. The surface of the agar was allowed to dry before streaking with standardized overnight broth cultures of our panel of bacteria strains. Plates were incubated at 37°C for 24 h under aerobic conditions. The MIC was defined as the lowest concentration of the antibiotic or extracts that completely inhibited visible growth of the test organism.

HPLC/DAD analysis for phenolic compounds: Phenolic compounds in the hydroalcoholic extract of *Artemisia vulgaris* were identified using HPLC coupled with Diode Array Detector (DAD) as described by Giner *et al.* (1993). Ten milligram of the plant sample was sonicated in HPLC graded methanol (1 mL) for 60 min. The resultant mixtures were centrifuged at 10000 g for 10 min and the supernatants was used for the HPLC/DAD analysis. The resulting solutions were filtered through a 0.22 μm filter and 10 μL aliquots of the filtrates were injected onto HPLC system. This system was composed of RP Nova Pack C18 column (300 \times 3.9 mm)

packed with 4 μm particles and a pre-column containing the same packing material. The columns were eluted in two solvent systems. Solvent A: water/phosphoric acid (0.1%) and solvent B: methanol. The solvent gradient was composed of A (75-0%) and B (25-100%) for 20 min, then 100% B for 4 min, then again at the initial conditions (75% A and 25% B) for 10 min. A flow rate of 1.0 mL min^{-1} was used at 30°C. Spectral data for all peaks were accumulated in the range of 285 nm using DAD (Gilson 170). Phenolic compounds were identified by comparison of retention times and spectral of each peak with those of known standards analysed under the same conditions.

MTT cytotoxicity assay: The cytotoxicity of AV extract was determined using MTT assay as described by Mosmann (1983) to measure cell viability. A volume of 100 μL from plant extract (15.62-500 $\mu\text{g mL}^{-1}$) were added in serial concentrations and then incubated for 24 h. After incubation, the medium was discarded and further incubated with 100 μL of tetrazolium dye (MTT) solution (0.5 mg mL^{-1} prepared in PBS) added to every well for an additional 4 h. The resulting dark blue formazan crystals formed due to mitochondrial activity after incubation was dissolved by adding 100 μL of DMSO into each well. The plate was then read on a microplate reader at 490 nm. MTT solution with DMSO (without cells and medium) acted as a blank control in microplate reading while the PBS-treated cells served as a control of 100% viability (Dua and Gude, 2006).

Statistics: Data analysis was done on microsoft excel to obtain descriptive statistics. Means were separated by the

Duncan multiple test using SAS. The different levels of significance within the separated groups were analysed using one way analysis of variance (ANOVA). Values were considered significant at $p < 0.05$.

RESULTS

The Total Phenolic Content (TPC) in the whole parts of *Artemisia vulgaris* extracted with 70% ethanol was assessed due to its diverse and strong therapeutic values in promoting human health. The TPC present in AV extract was expressed as Gallic Acid Equivalents (GAE) extrapolated from the standard curve $Y = 0.2421x$; $R^2 = 0.95641$. Here, the TPC in the extract recorded the value of $28.62 \pm 0.05 \text{ mg GAE g}^{-1}$ contrary to those reported elsewhere. Folin Ciocalteu Reagent (FCR) used for the assay has been indicated in most studies to measure other compounds and hence may not give a concise amount of the phenolic compounds present in the plant extract. Furthermore, the phenolic composition in the whole parts of *Artemisia vulgaris* was tentatively identified using HPLC-DAD an indispensable tool for chemical profile analysis. The identification of compounds was done by comparing the retention time and UV chromatogram with those of standards (Gallic acid and rutin) under the same chromatographic condition. Two main peaks of phenolic compounds were detected at 285 nm between the retention time of 2 and 80 min (Fig. 1).

The *in vitro* scavenging properties of AV extract or caffeic acid use as a reference drug against DPPH radical was determined via changes in absorbance at 517 nm. The antioxidative capacity of the extract was expressed as the

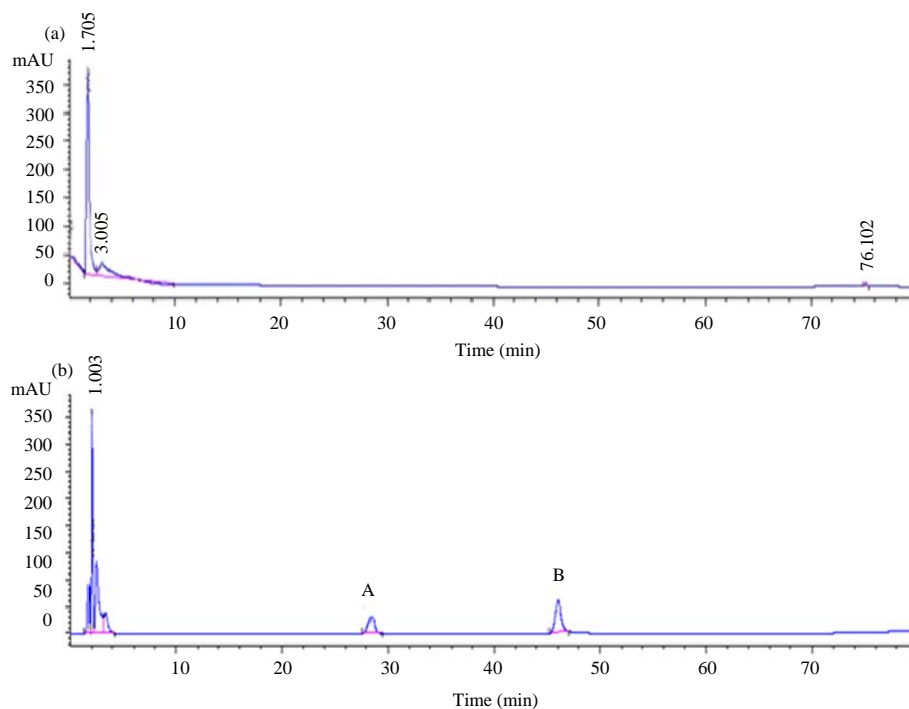


Fig. 1(a-b): HPLC-DAD Chromatograms showing peaks of standard phenolic compounds correspond to (A) Gallic acid and (B) Rutin, in comparison to the hydroalcoholic extract from *Artemisia vulgaris* (AV: 1 mg mL^{-1}) at 285 nm

IC₅₀ (µg mL⁻¹), an effective concentration at which the radical was inhibited by 50%, interpolated from the linear regression analysis. The AV extract demonstrated a moderate antioxidative activity against DPPH radical (IC₅₀: 976.0 µg mL⁻¹) as compared with caffeic acid (IC₅₀: 12.54 µg mL⁻¹), possibly due to its low phenolic content.

The formation of AGE (Advanced glycation end-products) was confirmed after 24 h incubation of BSA-glucose solution by measuring the fluorescence intensity. The observed increase in the glycation intensity was reduced after AV extract was added. The influence of the AV extract on glucose-induced glycation was concentration dependent, reduced AGE to 85, 75, 50, 35, 30 and 15% at 0.25, 0.5, 1.0, 2.5 and 5 mg mL⁻¹, respectively, having an IC₅₀ of 968.4 µg mL⁻¹ (Fig. 3). However, the percentage inhibition of AGE formation

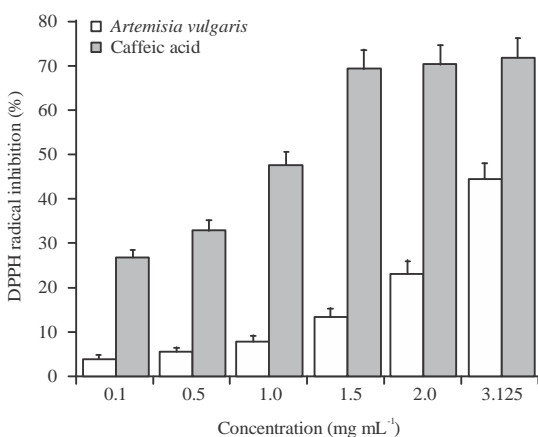


Fig. 2: DPPH radical scavenging capacity of hydroalcoholic extract from *Artemisia vulgaris* (AV; mugwort). Caffeic acid served as positive control for DPPH assay. The IC₅₀ of AV extract to scavenge DPPH radical was 976 µg mL⁻¹. Data is expressed as Mean±SD (n = 3)

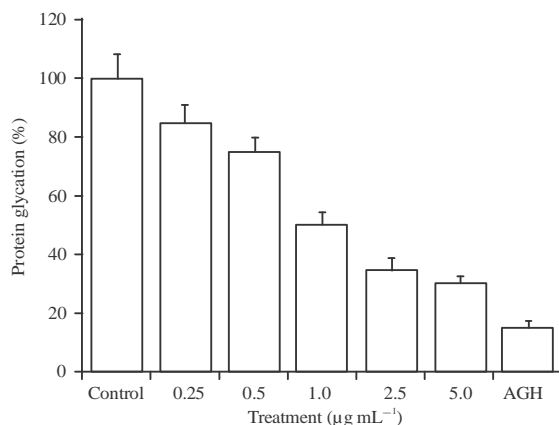


Fig. 3: Effect of hydroalcoholic extract of *Artemisia vulgaris* (AV; mugwort) and aminoguanidine (12.5 µg mL⁻¹) on BSA glycation. The IC₅₀ of AV extract against protein glycation was 968.4 µg mL⁻¹. Data is expressed as Mean±SD (n = 6)

by the extract was less active as compared to amino-guanidine (0.12 µg mL⁻¹: 12.5%), a known inhibitor of protein glycation process in diabetes subject.

The inhibitory potential of AV extract or caffeic acid against thiobarbituric acid reactive substances (TBARS) formation as lipid peroxide by-product in egg yolk is presented in Fig. 4. Additions of FeSO₄ to the egg yolk caused an increase in the TBARS formation but significantly reduced when react with the plant extract in a concentration dependent manner. The IC₅₀ values obtained for AV or caffeic acid (standard drug) was 0.36 and 0.074 mg mL⁻¹, respectively. The plant extract exhibited a moderate capacity to prevent MDA formation though remarkably (p<0.05) lower as compared with caffeic acid.

The antioxidative potential of AV extract against uric acid production from purine metabolism caused by xanthine oxidase was determined. The percentage inhibition of Xanthine oxidase (XOI) by the AV extract or standard drug is summarized in Fig. 5. A promising XOI activity was exhibited by the AV extract via reduction of uric acid generation with IC₅₀ value of 112.51 µg mL⁻¹ but less significant (p<0.05) as compared to the allopurinol a standard drug used clinically for xanthine oxidase inhibition (IC₅₀: 42.8 µg mL⁻¹).

The continuous trend in the use of medicinal plants as complementary and alternative therapy has necessitated further studies on their toxic nature for safety purposes. The toxic effect of this plant on HepG2 cells (Fig. 6). The extract demonstrated non-toxic effect to mammalian cells having an IC₅₀ value of 246.32±5.21 µg mL⁻¹, indicating no impairment on the mitochondrial viability. The result obtained was significantly (p<0.05) different as compared with the standard doxorubicin (5 µg mL⁻¹: 51.45%).

The antibacterial activity of *Artemisia vulgaris* extract against our panel of bacteria using agar dilution method is presented in Table 1. The plant extract exhibited mild to poor

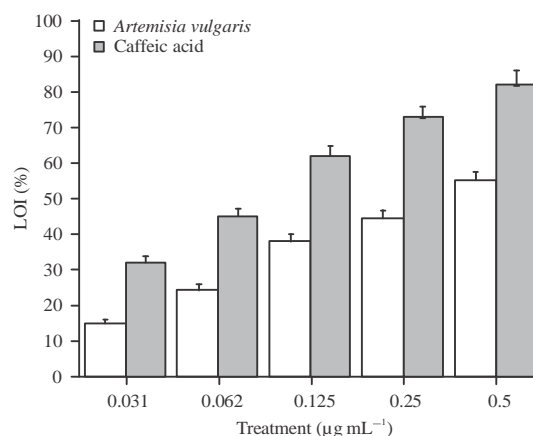


Fig. 4: Percentage inhibition of lipid peroxidation by the hydroalcoholic extract of *Artemisia vulgaris* (AV; mugwort) and caffeic acid. The LOI activity of the AV extract had IC₅₀ value of 0.36 mg mL⁻¹. Data is expressed as Mean±SD (n = 3)

Table 1: Antibacterial activity of hydroalcoholic extract of whole parts of *Artemisia vulgaris* (AV; mugwort) and streptomycin against selected clinical relevance bacteria

Bacteria	Gram +/-	MIC (mg mL ⁻¹)	Streptomycin (µg mL ⁻¹)
<i>Bacillus cereus</i> ATCC 10702	+	6.25	2
<i>Bacillus pumilus</i> ATCC 14884	+	>50	2
<i>Pseudomonas aeruginosa</i> ATCC 19582	-	6.25	8
<i>Serratia mercerscens</i> ATCC 9986	-	0.4	4
<i>Klebsiella pneumoniae</i> KZN	-	3.125	2
<i>Proteus vulgaris</i> ATCC 6830	-	0.4	4
<i>Enterobacter faecalis</i> ATCC 29212	+	0.4	2
<i>Acinetobacter calcoaconticus</i> UP	-	25	2
<i>Staphylococcus aureus</i> OK1	+	0.4	4
<i>Staphylococcus aureus</i> OK3	+	0.4	4
<i>Escherichia coli</i> ATCC 14884	-	>50	8

All MICs were determined in duplicate, MIC: Minimum inhibition concentration

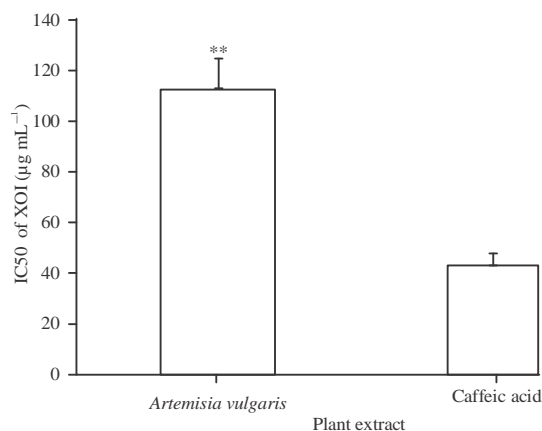


Fig. 5: Percentage inhibition of Xanthine oxidase by the hydroalcoholic extract of (AV; mugwort) and caffeic acid. Data is expressed as Mean±SD (n = 3). **p<0.05 as compared to standard caffeic acid. The IC₅₀ of 112.51 and 45.2 µg mL⁻¹ was obtained for *Artemisia vulgaris* extract and caffeic acid, respectively

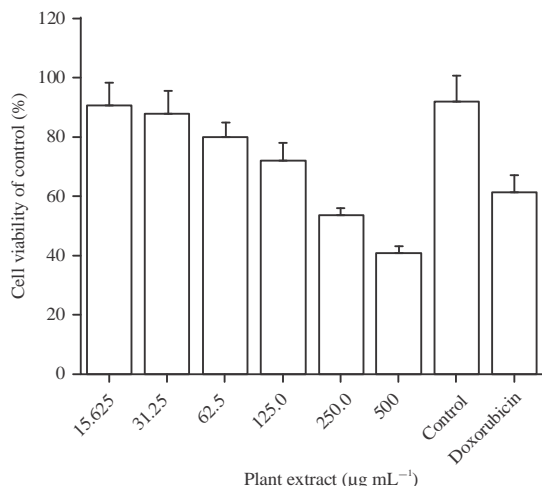


Fig. 6: MTT viability assay of HepG2 liver cells treated with hydroalcoholic extract from the hydroalcoholic extract of the whole parts of *Artemisia vulgaris*. Confluent cultures were treated for 48 h prior to MTT assay. Data is expressed as % control±SEM (n = 6). The IC₅₀ of the extract is 246.32±5.21 µg mL⁻¹

antibacterial activity with MIC values ranging between 0.4 and 50 mg mL⁻¹ whereas, the antibiotic of reference had MIC values ranging from 0.002-0.008 mg mL⁻¹. The bacterial strains: *P. vulgaris* ATCC 6830, *E. faecalis* ATCC 29212, *S. mercerscens* ATCC 9986, *S. aureus* OK1 and OK3 were more susceptible to the AV extract with MIC value of 0.39 mg mL⁻¹ while, other test bacteria were resistant having an MIC values ≥3.125. In this study, the plant extract was more active against gram positive bacteria than the gram negative bacteria.

DISCUSSION

Phenolic compounds are considered as major group of antioxidants due to their redox properties, OH groups, conjugated ring structures and carboxylic groups (Oyedemi and Afolayan, 2011). These compounds have played a significant pharmacological role in most chronic diseases associated with oxidative stress via hydrogen proton donation to the unpaired electron (Halliwell, 1999; Zhao *et al.*, 2008). There is increasing evidence that consumption of variety of phenolic compounds from plant sources may reduce the risk of serious health disorders. In this work, the TPC value recorded by the AV extract (28.04±0.05 mg GAE/g) is contrary to the values (217.46±2.30) reported by Erel *et al.* (2012) and (19.0±0.16 mg GAE/g) to that of Temraz and El-tantawy (2008). It could be inferred from this study that hydroalcoholic extract from the whole parts of *Artemisia vulgaris* had a low TPC value corresponding to the HPLC fingerprints and thus may be responsible for the mild antioxidative potential demonstrated in this work. HPLC-DAD is an indispensable tool for phenolic compounds analysis due to its versatility, precision and relatively low cost (Zhao *et al.*, 2008). We detected two peaks of phenolic compounds at the Retention Time (RT) of 3.095 and 76.102 min (solvent peak at RT 1.705 min) contrary to the 4 peaks (p-coumaric acid, ferulic acid, quercetin and kaempferol) detected by Ivanescu *et al.* (2010) using HPLC-DAD-MS, some of these peaks are missing in this work conceivably due to difference in methodology and solvent system used. It is apparent that AV extract is not a good source of phenolic compounds but may perhaps contain other antioxidant agents that are not phenolic in nature.

The DPPH is a stable nitrogen-centered free radical with violet colour though changes to yellow diphenylpicryl hydrazine via hydrogen or electron donor. This model has

been used globally to assess free radical scavenging and antioxidative capacities of plant-derived extracts. The obtained data is contrary to the findings of Temraz and El-tantawy (2008) who reported an IC_{50} value of 11.4 mg mL^{-1} of aqueous leaves extract from *Artemisia vulgaris* grown in Pakistan. This variation could be attributed to the degree of solvent polarity used for the extraction, the plant parts used and geographical location (Oyedemi *et al.*, 2013). Our data somewhat supported the ethno-therapeutic use of AV extract in combinatorial therapy for the prevention or treatment of diseases associated with oxidative stress.

Protein glycation occurs when the carbonyl group of a sugar reacts with an amino group of a protein to form a Schiff base, which rearranges to form a more stable Amadori product. It is one of the consequences of elevated blood glucose in diabetic subject that induces protein dysfunction resulting to several diabetic complications and age-related degeneration (Balu *et al.*, 2005; Kiho *et al.*, 2000). The ROS are generated during glycation and are able to diminish an oxidative defense of protein by decreasing thiol groups an indication of excess free radicals generation. In this study, glycation was generated based on the reaction between Bovine Serum Albumin (BSA) and high concentration of glucose. It has been shown that anti-glycation properties in most botanicals correlate significantly with their phenolic content (Hsieh *et al.*, 2007; Ho *et al.*, 2010). Consequently, the weak inhibition of AGE formation exhibited by the AV extract corresponds with its phenolic content and HPLC fingerprints.

Lipid oxidation is known as the major outcomes of free radical-mediated process, responsible for severe cellular malfunction (Dharmananda, 2003). It is important in the food deterioration and oxidative modification of low-density lipoprotein, which is now recognised as a key initial stage in the progression of arteriosclerosis (Noguchi and Niki, 1999). In this study, lipid peroxidation was initiated by the reaction between iron sulphate and egg yolk homogenate as lipid rich media. The observed data showed that AV extract had a mild polarity features to access lipid phase, where the lipid peroxidation process occurs to influence chain breaking reaction. This further established a mild potential of AV extract which may possibly due to its low phenolic compounds composition and that could justify the combinatorial therapeutic use of this botanical in folklore medicine.

Xanthine Oxidase (XO) is an important biological source of oxygen-derived radicals that contributes significantly to the oxidative damage of biomolecules (Valko *et al.*, 2007). It has been implicated in uric acid deposition ensuing in painful inflammation particularly in renal failure (Forbes *et al.*, 2008). Presently, allopurinol is the only clinical approved drug for XO inhibitor but limited in use due to its adverse effects such as hepatitis, nephropathy and allergic reactions (Nakamura, 1991; Fagugli *et al.*, 2008). These limitations have invigorated the quest for validating the efficacy of medicinal plant for xanthine oxidase inhibition. A promising XO activity was displayed by the extract but warrant further studies to identify or isolate the active principle responsible for this activity which could be harnessed for human benefit in the treatment of disease associated with uric acid deposition in human tissues.

It is generally assumed that most medicinal plants are safe and without toxic effect to human cells however studies have revealed that some plant extracts or active ingredients had diverse side effects and toxic in experimental animal model (Lutterodt *et al.*, 1999). Additionally, there is an increasing report of negative health consequences from the patients that use herbal medicines (WHO, 1991). Paradoxically, tens of thousands of people every year turn to herbal medicine as being free from undesirable side effects. Consequently, the safety of AV extract as herbal remedy in traditional medicine was tested in this study. The MTT is a rapid and high accuracy colorimetric approach currently in use to determine cytotoxicity of natural products for new drug development. The assay measured integrity of cell membrane by determining mitochondrial activity via enzymatic reduction of MTT to formazan (Houghton *et al.*, 2007). Our data showed that the plant extract had no toxic effect to human cells, suggesting there is no impairment to mitochondrial viability and thus support its safe use as a complementary and alternative herbal therapy.

The plant extract was more active against gram positive bacteria than the gram negative bacteria in accordance to several investigations. The poor antibacterial activity exhibited by AV extract concurred with the findings of Hiremath *et al.* (2011) who revealed an MIC of 25 mg mL^{-1} of ethanol leaves extract from *Artemisia vulgaris* towards *Escherichia coli* and *Staphylococcus aureus*. The high resistance depicted by the gram negative bacteria is already known due to the hydrophilic nature of their outer membrane that restricts penetration of the extract active components. It is interesting to note that *B. pumilus* ATCC14884 a gram positive bacterium displayed a strong resistance to the extract treatment, the mechanism which could not be explained, but may possibly due to its spore formation (Benardini *et al.*, 2003). Our observation strongly implies that *Artemisia vulgaris* can be an effective herbal remedy in the treatment of human infections caused by *P. vulgaris* ATCC 6830, *E. faecalis* ATCC 29212, *S. mercescens* ATCC 9986 and *S. aureus* OK1 and OK3.

CONCLUSION

We showed that hydroalcoholic extract from the whole parts of *Artemisia vulgaris* had a mild antioxidative capacity which is evident in DPPH radical scavenging, xanthine oxidase and protein glycation as well as lipid peroxide inhibition. A selective antibacterial activity was demonstrated against gram negative and gram positive bacteria. The extract was not toxic to the liver carcinoma cells and thus justifies its safe use as a complementary therapy in herbal medicine. Further studies *in vivo* are needed to justify its use in folklore and traditional medicine.

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