ORIGINAL RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Phytochemical and antibacterial evaluation of ethanolic extract of *Salvadora persica* root extract against selected microorganisms

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Received for publication: September 23, 2015; **Accepted**: October 22, 2015

Abstract: The aim of this paper was to anlyse phytochemical constituents and antibacterial activity of ethanolic extract of *Salvadora persica* roots against selected microorganisms. Organism tested were *Proteus vulgaris, Escherichia coli, Salmonella typhi, Bacillus cereus and Enterobacter aerogenes.* From the study *Salvadora persica* roots was found to contain secondary metabolites such as alkaloids, flavanoids, glycosides, saponins and terpenes. The plant extract inhibited the growth of *Enterobacter aerogenes* with a zone of inhibition of 32.833 ± 0.167, *Escherichia coli* (32.166 ± 0.167), *Bacillus cereus* (31.533 ± 0.033), *Proteus vulgaris* (31.166 ± 0.167) and *Salmonella typhi* (27.500 ± 0.289). The bioactivity of the plant extract observed in this study can be attributed to the presence of the mentioned phytochemicals. Results from the bioassay *of Salvadora persica* roots extract showed the highest zone of inhibition was obtained against *Enterobacter aerogenes* at 500 mg/ml. The paper concludes that *Salvadora persica*roots contain phyto medicine which is effective in treating most of the infectious diseases.

Key words: Salvadora persica; phytochemical; antibacterial; medicinal herbs; root; sethanolic

INTRODUCTION

In continuation of our interest in biological activities of natural medicinal plants extracts (Anthoney *et al.,* 2013; Anthoney *et al.,* 2014; Anthoney *et al.,* 2015 and Obey *et al.,* 2014), we report here the antibacterial activities of ethanolic extract of *Salvadora persica*roots. Decoction of the *Salvadora persica* root is used to cure gonorrhoea, spleen trouble, anthelmintic, malaria and general stomach ache. Roots also used for chest diseases, or they are pounded and used as a poultice to bring boils to a head. Root decoction also used to increase lactation in Maasai mothers. The bark is scratched and the latex used for treating sores. The young stem is widely used as a toothbrush and is believed to cure gun diseases. Dried bark decoction drunk with strong tea for fever and colds. Root decoction given to cattle with anthrax or as an emetic (Kokwaro, 2008).

Salvadora persica is traditionally used by the Maasai community as a medicinal plant to treat various diseases as well as lactating mothers to increase the amount of milk for the baby. It is used for centuries as a natural toothbrush; its fibrous branches have been promoted by the World Health Organization for oral hygiene. The plant has been used for the preparation of a number of medicinally important products such as abrasives, antiseptics, astringent, detergents, enzyme inhibitors and fluorides. *Salvadora persica* is used traditionally in the treatment of rheumatism, leprosy, gonorrhea, ulcers, scurvy, tumors and dental diseases (Al-Ali F, 2003). These diseases have caused many deaths and others like gonorrhea has led to infertility among females and even sometimes male. Ulcers like stomach ulcers may cause people to be allergic to some foods which may lead to unhealthy life. *Helicobacter pylori* bacteria eat the stomach lining thus causing ulcer by exposing the stomach to the digestive juices. It leads to the coughing of the blood, headaches and diarrhea.

Salvadora persica is a slow growing, evergreen perennial halophyte capable of growing under extreme conditions, from very dry environments to highly saline soils. It is a shrub or a small tree which grows up to 10 meters in height and a girth of 3 feet. Main trunk is erect or trailing, more than one foot in diameter, with profusely branched, wide crown of crooked, straggling and drooping branches. Young branches are green in colour. Bark is slightly rough, grayish brown on main stem, paler elsewhere. Leaves are opposite, entire, succulent, petiolated, fleshy, oblong elliptic to almost circular, 3x7cm, light to dark green, with 5-6 pairs of main nerves. New leaves are produced during April, which on maturity become thick and leathery. Leaves shed from late December to January (Janda, 2006).

Flowers are small, greenish yellow in axillary and terminal panicles, sessile or sub-sessile, bisexual and tetramerous. Small greenish-white flowers are produced in January to April. The fruit is yellow and ripens in the months of May and June. Mature fruits are spherical or globose drupe with persistent calyx, smooth, fleshy, 5-10 mm in diameter, pink to scarlet and single seeded. Seeds turn from pink to purple-red and are semitransparent when mature. Seeds are dispersed by birds, animals and man after they eat the fruit. Furthermore, S. species are deep rooted mesomorphic xerophytes as well as facultative halophytes with high salt tolerance (Sanders, 1997).

The genus *Salvadora* belongs to family *'salvadoraeceae'*. It comprises three genera (i.e. *Azima*, *Dobera*and *Salvadora*) and 10 species distributed mainly in the tropical and subtropical region of Africa and Asia.

The taxonomic position (Patricia, 2012) of *Salvadora persica* is as follows:

Binomial name: *Salvadora persica*
Kingdom: *Plantae*
Division:*Magnoliophyta*
Class: *Magnoliopsida*
Order: *Brassicales*
Family: *Salvadoraceae*
Genus: *Salvadora*
Species: *persica*

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The plant is named in different languages as below:

English: Salt Bush tree, Tooth Brush tree
Hindi:Chotapilu(salt bush)
Maasai: Oremit
Taita:Kizungumotp
Turkana:Esekon
Gogo: Mkunguni
Swahili: Mswaki
Giriama: Muezamoyo,

Salvadora persica is found at an altitude of 1800 meters. It is widespread in arid regions, on saline lands and in coastal regions, thorn shrubs, desert flood plains, and grassy savannahs. It prefers areas such as riverbanks, on perimeters of waterholes, along drainage lines in arid zones and in seasonally wet sites where ground water level is high indicating its tolerance to a wide range of water, soil and soil pH conditions and that is probably the main reason for its widespread nativity. The plant is also found in valleys, on dunes and on termite mounds (Majowicz, 2010).

Its annual rainfall requirement is 300-1000 mm. The tree is able to tolerate a very dry environment with mean annual rainfall of less than 200 mm. It prefers clays but also found on loam, black soils and sand. It is adapted to alkaline or very saline soils, usually clay rich, and soils without salt. *Salvadora persica* showed some variations in its distributional behavior in different countries, which may be attributed to changes in water resources, climatic factors, edaphic variables and anthropogenic pressures along the elevation gradient. It is native to Algeria, Egypt, India, Nigeria, Pakistan, Saudi Arabia, Sri Lanka, Uganda, Kenya and Zimbabwe (Heymann, 2008).

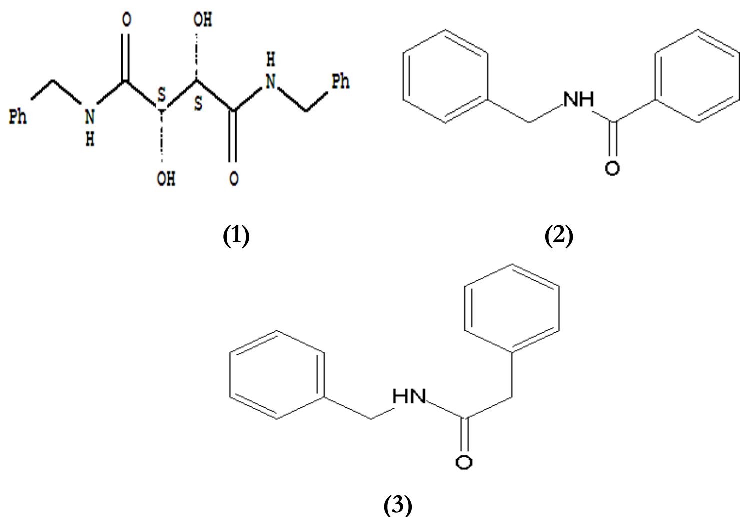
Fresh bark is used as a vesicant. Root decoction is used against gonorrhea and vesical-catarrh. Root extract is used to relieve the pain due to spleen troubles. Bark decoction used as a tonic and stimulant in low fevers. Leaves are used in treatment of asthma, cough and piles. Fruits possess carminative and diuretically properties and used in treatment of rheumatisms (Abdel-Wahab, 1990). Keeping the fact that roots of *Salvadora persica* can treat some diseases an attempt was made to screen the antimicrobial nature of *Salvadora persica* extracts against some pathogenic bacteria and fungi (Mansy, 2001). It is used for centuries as a natural toothbrush; its fibrous branches have been promoted by the World Health Organization for oral hygiene. The plant has been used for the preparation of a number of medicinally important products such as abrasives, antiseptics, astringent, detergents, enzyme inhibitors and fluorides (Janda, 2006).

Salvadora persica is used traditionally in the treatment of rheumatism, leprosy, gonorrhoea, ulcers, scurvy, tumours and dental diseases. It has been suggested that antimicrobial substances that naturally protect plants against various invading microorganisms or other parasites may leach out into the oral cavity and that these compounds may benefit the users by protection against carcinogenic and periodontopathic bacteria (Heymann, 2008).

*Salvadora persica*is known to contain several biologically active chemical constituents such as volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and carbohydrates. Almost every part of the plant has pharmaceutically important ingredients. The leaves, roots and stem bark contain an alkaloid trimethylamine (Batz, 2011). The seed is rich in oil and contains lauric, myristic and palmitic acids. Its oil has high potential for making soaps, candles and to be used as a substitute for coconutoil. The root contains elemental gammamonoclinicsulphur, benzyl glucosinolate, salvadourea (aurea derivative), m-anisic acid and sitosterol. Benzyl isothiocyanate which is isolated from the root, exhibits antiviral activity against Herpes simplex virus-1 which affects oral region (Scallan, 2011).

Studies have found that *Salvadora persica* extracts and volatiles contained one major antibacterial component, benzyl isothiocyanate (BITC), with rapid bactericidal effect against all Gram-negative bacteria including periodontal pathogens, but low effect on Gram-positive bacteria. Electron microscopy revealed that the bacterial envelope was severely damaged by both *Salvadora persica* extracts as well as commercial pure BITC. Extracts from *Salvadora persica* root might thus be an avenue to explore for applications as an adjunct to treatment of periodontal diseases. The strong and rapid killing affected exclusively Gram-negative bacteria, including medically important pathogens such as *Salmonella enterica, Pseudomonas aeroginosa* and *Haemophilus influenza* (Heymann, 2008).

According to chemical and phytochemical analysis of *Salvadora persica*, its stem yielded octacosanol, 1-triacantanol and â-sitosterol. Onexamination by thin layer chromatography, it was found to be a mixture of two compounds, which were separated by column chromatography. Compound **A** had molecular formula $C_{29}H_{50}O$ (C = 83.75%, H = 12.25%) and compound **B** was found to be the white crystalline compound, with the molecular formula $C_{35}H_{60}O_6$, (C = 72.9%, H = 14%).Benzylamide were also isolated. The isolated compounds were identified as butanediamide, *N*1, *N*4-*bis* (phenylmethyl)-2(*S*)-hydroxy-butanediamine (1), *N*-benzyl-benzamide (2), *N*-benzyl-2- phenylacetamide (3), and benzyl urea (4). *N*-benzyl-2- phenylacetamide revealed a significant inhibitory effect on human collagen-induced platelet aggregation, and a moderate antibacterial activity against *Escherichia coli* (Leon, 1950).



(1) (2)

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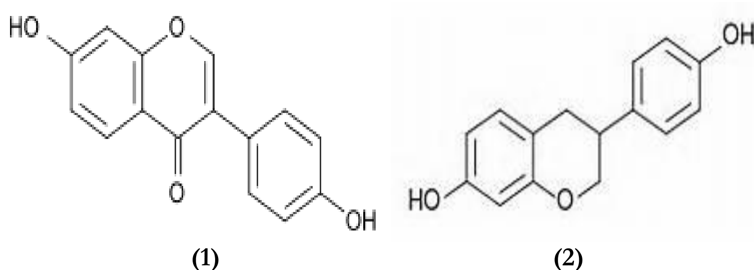
Bacteria are everywhere; in soil, in water, in air, and in the bodies of every person and animal. Most Bacteria are harmless, or even essential to life however, some bacteria are harmful to life. Nitrate is generally considered a water pollutant and undesirable fertilizer residue in the food chain. By reducing nitrate to nitrite, commensal bacteria might be involved in the pathogenesis of gastric cancer and other malignancies, as nitrite can enhance the generation of carcinogenic N-nitrosamine (Nataro, 1998).

Fructooligosaccharides are produced from sucrose with the aid of β- fructofuranisidase from *Aspergiliusniger* on a commercial scale. It has been found that they are not hydrolyzed by any digestive enzyme of humans and animals. The fructooligosaccharide are selectively utilized, particularly by bifidobacteria. The clinical study shows that fructooligosaccharide administration improves the intestinal flora with subsequent relief of constipation, improves blood lipids in hyperlipidemia, and suppressed the production of intestinal putrefactive substances (Jeyachandran, 2007).

Live bacteria that survive passage through the gastrointestinal tract and have beneficial effects to the host, like probiotic bacteria has been found to treat diarrheal disease, prevents cancer or the formation of carcinogens, lowers the serum cholesterol and stimulates the immune system, (Agyare, 2013). Diadzein[**1**], and isoflavonephytoestrogenp[**2**] found in soy, is metabolized to equol[**3**] and o-desmethylangolenism(O-DMA) by intestinal bacteria. Observation and intervention studies in humans have suggested that the ability to produce equol and O-DMA may be associated with reduce risk of certain diseases including breast and prostate cancer (Taye, 2011).



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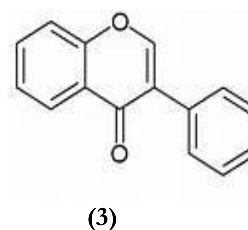

(3)

The *in vitro* fermentability of oligo fructose and inulin was studied by measuring bacterial end-product formation in batch culture. Short chain fatty acid and gas formation indicated that substrates which occur naturally in the diet and reach the colon in a largely intact form were utilized by mixed populations of gut bacteria. These bacteria exerted a preferential stimulatory effect on numbers of the health- promoting genus bifidobacterium, whilst maintaining population of potential pathogens (*Escherichia coli*) at relatively low levels. An increase in the concentration of the substrate in the diet may therefore improve the composition of the large intestinal microflora and have positive effects on the quality of the western diet(Almas,1999). A study done to compare the ability of several strain of Lactic Acid Bacteria(LAB) to modulate Cytokine secretion by human intestinal epithelial cell (IEC) line HT-29. Certain strains of the bacteria suppressed the production of the chemokine RANTES by stimulated HT-29 IEC. Strains-dependent effects were also seen for the suppression of the tumor necrosis factor and transforming growth factor production. Modulation of IEC Cytokine production has the potential to profoundly affect the mucosal microenvironment, influencing the immune response to pathogens and other ingested antigens (Almas, 2000).

Streptococcus pneumonia is a human pathogenic bacterium which causes pneumonia. It resides asymptomatically in the nasopharynx of healthy carriers. The respiratory track, sinuses and nasal cavity are the parts of the host body that are usually infected. It is the main cause of community acquired pneumonia and meningitis in children and the elderly, and of septicemia in HIV-infected persons (Abdel-Wahab, 1990).

Salmonella typhi is a rod-shaped, gram negative bacterium which has a complex regulatory system and meditates its response to the change in its external environment. In order to survive in the intestinal organs of its host where there are low levels of oxygen it uses electrons acceptors like nitrogen. It passes through the lymphatic system of the intestine into the blood of the patient's and is carried into various organs. *Salmonella typhi* is a food born pathogen, which has killed over 600,000 people annually all over the world. It is a deadly bacterial disease that causes typhoid fever and is transmitted through food and water (Khalil, 2006).

Whooping cough is a disease caused by a bacterium called *haemophillus pertussis.* It is an aerobic, non-spore forming, gram-negative *ccoccobacillus* bacterial pathogen that is strict to infecting and residing in the mouth, nose and throat of humans. Transmission occurs via airborne respiratory

droplets from other people's cough and sneezes. About 48.5 million yearly cases are reported worldwide and about 295,000 deaths. Research show that the disease occurs among female more than male and claims that the disease occurrence is related to seasons and geographical areas (Galati, 1999).

The present study was carried out to evaluate the phytochemical and antibacterial activity of ethanolic extract of *Salvadora persica* leaves against selected pathogenic organisms.

Materials and Methods

Preparation and Extraction

The dry roots were crushed using a grinder and the crushed roots particles were weighed in an analytical balance to determine mass. Two hundred and twelve grams (212g) of the powdered roots were mixed with 400ml of ethanol – water (90:10). The mixture was kept for twenty-four hours (24hrs) on a shaker for effective extraction of the plant components. The mixture was then transferred for filtration where the filtrate was separated from the residue using a filter paper in sanction pump. The residue was reserved and the filtrate obtained was taken for extraction process in a rotary evaporator machine. The extraction process took about eight hours for the extract to dry in about 40^0C and the solvent evaporated completely. The crude extract obtained which was fourteen grams (14g), was kept in a freezer for about twenty-four hours at 4^0C before Bioassay activity.

Phytochemical analysis

Chemical test was carried out on the crude extract of *Salvadora persica* using standard procedure to identify the presence of a certain phytochemical constituents present in a plant. The screening of phytochemical constituents of *Salvadora persica* was performed using generally accepted laboratory technique for qualitative determinations.

Test for tannins: One gram of sample was boiled with 20 ml distilled water for five minutes in a water bath and then filtered while hot. About 1 ml of cool filtrate was distilled by using 5 ml of distilled water and a few drops (2-3) of 10 % ferric chloride were observed for any formation of precipitates and any colour change. A bluish-black or brownish-green precipitate indicated the presence of tannins.

Test for saponins: One gram of extract was boiled with 10ml of distilled water in a water bath for 10minutes. The mixture was filtered while hot and allowed to cool. The following tests were then carried out.

- **Demonstration of frothing:**2.5 ml of filtrate was diluted to 10ml with distilled water and shaken vigorously for 2minutes (frothing indicated the presence of saponin in the filtrate).
- **Demonstration of emulsifying properties:** 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water (above), shaken vigorously for a few minutes (formation of a fairly stable emulsion indicated the presence of saponins).

Test for terpenoids and sterols (Liberman-burchard test): Five gram of the extract was mixed with approximately 30ml of ethanol and is then boiled. The mixture was filtered into test tubes. The residue is extracted with dimethyl ether and transferred to spot plate and allowed to dry. 3 drop of acetic anhydride was added and stirred. 1 drop of concentrated sulphuric acid was added to the wall of spot plate to allow them to mix slowly. Any formation of colour is observed. A reddish brown precipitate colouration at the interface formed indicated the presence of terpenoids.

Test for flavonoids: One gram of the extract was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

Test for alkaloids: One gram of the extract was boiled with water and 10 ml hydrochloric acid on a water bath and filtered. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of the following reagents will be added to about 0.5 ml of the filtrate in a test tube and observed. Picric acid solution, 10% tannic solution and Mayer's reagent (Potassium mercuric iodide solution). The test tubes were observed for coloured precipitates or turbidity. The formation of the precipitate indicated the presence of alkaloids.

Test for cardiac glycosides: Five ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

Bioactivity analysis

Preparation of the Bacterial Suspension: The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard. The McFarland standard was prepared by dissolving 0.5 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). Sulphuric acid (1%) was prepared in a 100-ml volumetric flask. McFarland Standard, 0.5 ml of the 1% $BaCl_2$ solution was mixed with 99.5 ml of H_2SO_4 solution. The turbidity of the 0.5 McFarland Standards was measured with the aid of a spectrophotometer at a wavelength of 625nm to read an optical density of between 0.08-1.0. At this absorbance, the McFarland Standard represents a bacterial cell density of approximately 1.5 x 108 CFU/ml (1.0 x108 – 2.0 x 108 CFU/ml. It was then transferred to a screw-capped bottle and sealed with parafilmto prevent evaporation due to exposure to air. The bacterial suspensions were then tested against the McFarland standards until they reached the absorbance of the McFarland standard and then they were ready for use.

Preparation of the Extract Concentrations and Antibiotic: Stock solutions for the extract were prepared by dissolving 500mg in1ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 1µg of penicillin in

1ml of sterile distilled water. DMSO served as a negative control.

Screening for the antibacterial potential of the plant extract: The agar well diffusion procedure used in the experiment was similar to that used by (James,2004). The microorganisms used for this study were laboratory strains of Proteus *vulgaris, Escherichia coli, Salmonella typhi, Bacillus cereus* and *Enterobacter aerogenes*. A single colony for each of the organisms was picked from agar plate and dissolved in 5 ml of Mueller Hinton broth. The broth was incubated overnight at 37^0C. Five (5) ml of plain Mueller Hinton broth was incubated alongside the organisms to ensure that the medium was not contaminated. The spectrophotometer was set to 625 nm wavelength and each of the microbial cultures was pipetted into cuvettes to measure the absorbance. A cuvette of plain Mueller Hinton broth was used a blank at 0.000 absorbance. The absorbances of the microorganisms were measured. The bacterial organisms exceeding 0.1absorbance or falling below 0.08 absorbance were adjusted by adding bacterial suspension until the absorbance fell between 0.08-0.10, matching the McFarland Standard. One hundred (100) µl of each of the organism were then inoculated onto agar plates for the bioassay(Hassan,2011). Three 6mm wells were made into each agar plate using a sterile metal corkborer. 100µl of the standard drug penicillin was placed in one well, the extract in another well and dimethylsulfoxide(DMSO) was placed in the third well on each plate. The experiment was run in triplicate for each extract and each organism tested. The plates were incubated for twenty-four to forty-eight hours and the zones of inhibition were measured in millimeters with the aid of a meter rule.

RESULTS AND DISCUSSION

Table 1: preliminary phytochemical of *Salvadora persica roots extract*

Plant constituents	Chemical test	Result	Descriptive results
Alkaloids	Mayer's test	+	Formation of slight opaqueness
Flavanoids	Bate-Smith and Metcalf test	+	Formation of magenta red colour
Glycosides	Fehling's test	+	Formation of brick red precipitate
Saponins	Froth test	+	Formation of froth
Taninswa	Ferric chloride test	–	No blue black and brownish-green colour
Terpenes	Liebermann-Burchard test	+	Formation of red colour
Sterols	Liebermann-Burchard test	–	No formation of blue colour

+ indicates presence and – indicates absence

In this experiment, we determine phytochemical compounds through the screening of medicinal plants. In alkaloid test the plant material is first grind off with pestle and mortar to increase surface area for the process of extraction of the phytochemical compounds. The acid wash sand is used as an abrasive so that the tough plant material can be easily extracted. Chloroform and ammoniacal chloroform are used for the extraction of active ingredients (alkaloids) in this plant. The sulphuric acid is added to separate chloroform layer. The plant extract shows precipitate form (slight opaqueness). The results shows that the plant has alkaloid. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Some even reported for its analgesics, antispasmodic and antibacterial property (Sofowra, 1993).

Flavonoids are secondary metabolites with polyphenolic structure and synthesized in plants, through polypropanoid pathway (Ali, 2011). Flavonoids form part of the largest category of phytochemicals, the phenolic phytochemicals. The term 'phenol' encompasses a variety of plant compounds containing an aromatic ring with one or more hydroxyl groups. Many phenolic occur in nature with a sugar group attached a thesis, making them water soluble. Flavonoidsare present as shown by the formation of magenta red solution. Dietary flavonoids represent a diverse range of polyphenolic compounds that occur naturally in plant foods. The range and structural complexity of flavonoids has led to their sub-classification as flavonols, flavones, flavanones, flavan-3-ols (and their oligomers, proanthocyanidins), isoflavones and anthocyanins. They are present in significant amounts in many commonly consumed fruits, vegetables, grains, herbs and drinks, these structurally diverse compounds exhibit a range of biological activities in *vitro* which may explain their potential cardio protective and anti-cancer properties, including antioxidant, anti-inflammatory, apoptotic activity and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Sharma, 2006).

The extract also yielded positive with terpenes as indicated by the presence of red color in solution in Liebermann-Burchard Test. Terpenoid in plants are usually used for their aromatic quality. It is reported that terpenoid has used in traditional herbal medicine for anti-bacterial, anti-neoplastic and other pharmaceutical functions. Steroids are widely used for dietary fat, sex hormones and the anti-inflammatory (Just *et al*, 1998).

Saponin testing indicate the presence of saponin in the plant sample which is weakly positive with little froth presence in it. This error may indicate that we have added too much of the distilled water to a point that the saponin is not enough to produce froth. May be the extract of saponin in the leaves are not enough for the test. Saponin is known to produce inhibitory effect on inflammation. It coagulates and precipitates the blood cells (Okwu *et al.,* 2006). They have been found to treat hyper cholestrerolemia, hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anti-cancer and weight loss (Moabe, 2013).

The results show that the plant has no tannins. Tannins differ from the phenolic in that they are compounds of high molecular weight. They are highly hydroxylated and can form insoluble complexes with carbohydrates and proteins. The term 'tannin' is derived from its tanning properties; it forms stable tannin protein complexes in animal hides, as in leather. Glycosides are secondary metabolites which are organic compounds from plants or animal sources in which a sugar is bound to non-

carbohydrate moiety. Cardiac glycosides have been used as arrow poisons or heart drugs. There was a presence of glycosides as manifested by the formation of brick red precipitate when the extract was subjected to Fehling's Test. The unexpected results relating cardiac glycosides with anticancer properties have created a great interest in glycosides. This has led to clinical trial of cardiac glycosides based drugs in clinics (Newmann, 2008). The presence of these phytochemicals in *Salvadora persica* roots extract is an indication that *Salvadora persica* has curative effects and therefore can be used as alternative medicine.

Table 2: Zone of inhibition (mm ± S.E.) of ethanolic extract of *Salvadora persica* roots against selected bacterial organisms

Microorganism	Zone of Inhibition (mm ± S.E.)	Penicillin Control	DMSO control
Proteus vulgaris	31.166 ± 0.167	39.50 ± 0.289	0.00 ± 0.000
Escherichia coli	32.166 ± 0.167	38.50 ± 0.289	0.00 ± 0.000
Salmonella typhi	27.500 ± 0.289	29.50 ± 0.289	0.00 ± 0.000
Bacillus cereus	31.533 ± 0.033	40.333 ± 0.167	0.00 ± 0.000
E. aerogenes	32.833 ± 0.167	39.000 ± 0.000	0.00 ± 0.000

Key: S.E. = Standard error; DMSO = Dimethylsulfoxide

The average mean zone of inhibition (±S.E.) was calculated for each of the microbial organism. The zones of inhibition of the microorganisms were also analysed by analysis of variance (ANOVA) and it was shown that there were significant differences in the zones of inhibition among the microbial organisms ($p<0.05$). The biggest zone of inhibition was against *Enterobacter aerogenes* (32.833 ± 0.167) followed by *Escherichia coli* (32.166 ± 0.167), *Bacillus cereus* (31.533 ± 0.033), *Proteus vulgaris* (31.166 ± 0.167) and *Salmonella typhi* (27.500 ± 0.289). The study shows that the ethanolic extract of *Salvadora persica* roots can inhibit the growth of all the five microorganisms against (Table 1). All the extracts were significantly smaller in zones of inhibitions than the positive controls except *Salmonella typhi* control. Comparing for the mean zones of inhibition of 500mg/ml of *Salvadora persica* roots showed that there was significant difference in the zones of inhibition among the organisms $p<0.001$.

Table 3: Tukey's honestly significant differences between microorganisms treated with the ethanolic extract of *Salvadora persica* roots and antibiotic control.

Comparison	P- Value	Significance
Proteus vulgaris vs Escherichia coli	**0.073**	**NS**
Proteus vulgaris vs Salmonella typhi	**0.000**	**S**
Proteus vulgaris vs Bacillus cereus	0.958	NS
P. vulgaris vs Enterobacter aerogenes	0.001	S
Proteus vulgaris vs P.vulgaris control	0.000	S
Escherichia coli vs Salmonella typhi	0.000	S
Escherichia coli vs Bacillus cereus	0.534	NS
Escherichia coli vs Enterobacter aerogenes	0.468	NS
Escherichia coli vs E. coli control	0.000	S
Salmonella typhi vs Bacillus cereus	0.000	S
Salmonella typhi vs E. aerogenes	0.000	S
Salmonella typhi vs S. typhi control	0.000	S
Bacillus cereus vs E. aerogenes	0.000	S
Bacillus cereus vs B. cereus control	0.000	S
E. aerogenes vs E. aerogenes control	0.000	S

Key: **NS** = Not Significant; S= Significant

On further comparison using the Tukey's pairwise (**Table 3**) comparison showed that zones of inhibition for *Proteus vulgarisis* significantly bigger than *S. typhi* and smaller than *E. aerogenes* and their positive control ($P < 0.05$). The zone of inhibitions of P. *vulgaris* was not significantly different from those of *E. coli* and *B. cereus* ($P > 0.05$). The zone of inhibitions of *E. coli* was not significantly different from that of *B. cereus* and *E. aerogene* but significantly higher than *S. typhi* and smaller than their control. The zones of inhibition of *S. typhi* were significantly smaller than those of microorganisms such as *B. cereus*, *E. aerogene* and their positive control. The zone of inhibitions of *B. cereus* was significantly smaller than microorganism *E. aerogenes* and their antibiotic control. The zone of inhibition of *E. aerogenes* was significantly smaller than their positive control. However, there was no zone of inhibition produced by the negative control DMSO against any of the microorganisms.

According to Atef (2013), the highest zones inhibition was obtained for *Streptococcus strains*with a ratio of inhibition 10, 80 and 100% at 12.5, 25 and 50% concentration of (AMRE 24). While, inhibition ratios were 50, 100 and 100% at 12.5, 25 and 50% concentration of (AMRE 48), respectively. There was no inhibitory effect at 12.5% concentration of (AMRE 24) for *Bacillus sabtilis, E.coli, Salmonella typhimurium* and *Candida albicans*. The most resistant bacterial strain was Pseudomonas aeruginosa which was not affected with all treatments except 50% of (AMRE 48) exhibiting 50% growth inhibition. *Bacillus sabtilis and Candida albicans* showed resistance at 12.5% concentration of both (AMRE 24) and (AMRE 48).

The microorganisms used for this study was laboratory strains of *Proteus vulgaris, Escherichia coli, Salmonella typhi, Bacillus cereus* and *Enterobacter aerogenes. Proteus vulgaris* meningitis is relatively uncommon. It may occur by direct extension from an adjacent otitis media or mastoiditis and occasionally as a complication of septicemia from a focus of infection, usually in the genito-urinary tract. The causative organism is a gram-negative, aerobic, non-sporulating, actively motile bacterium, usually occurring as a saprophytic non-pathogen in the upper respiratory, gastrointestinal, or genito-urinary tracts. Occasionally it produces severe infection and death (El-Ghonemy, 1993). *Proteus* species are the major cause ofdiseases acquired outside the hospital, where many of these diseases eventually require hospitalization (Darmani, 2006). *P. mirabilis* causes 90% of Proteus infections. *Proteus* species, particularly *P. Mirabilis*, is believed to be the most common cause of infection-related kidney stone, one of the most serious complications of unresolved or recurrent bacteruria (Liblikas, 2005). *P. mirabilis* has been implicated in meningitis, empyema, osteomyelitis and gastroenteritis. Also, it frequently causes nosocomial infections of the urinary tract (46%), surgical wounds (24%) and lower respiratory tract (30%). Less frequently, *proteus*species cause bacteraemia (17%), most often in elderly patients (*Mathur et al*, 2002).

Bacillus cereus in association with food poisoning and eye infection, recognition and appreciation for the multitude of other serious infections such as fulminant sepsis and devastating central nervous system infections are lacking. The suspicion of the association of *B. cereus* with these mounting infectious complications moves with a fatal

lethargy in its recognition as a bona fide human pathogen. Clinicians and clinical microbiologists must both give serious consideration to the significance of a *B. cereus* isolate from a clinical specimen, especially if the patient is immunosuppressed (Alshammary*et al*, 2008).

Enterobacter aerogenes is a Gram negative, catalase positive, indole negative, rod shaped bacterium (Batwa, 2006). It is a nosocomial and pathogenic bacterium that causes opportunistic infections including most types of infections. The majority is sensitive to most antibiotics designed for this bacteria class, but this is complicated by their inducible resistance mechanisms, particularly lactamase which means that they quickly become resistant to standard antibiotics during treatment, requiring change in antibiotic to avoid worsening of the sepsis. *Enterobacter aerogenes* causes disease in humans through inadvertent bacteria transfer in hospital settings. A selection of enteric bacteria like *E.aerogenes* is opportunistic and only infects those whoalready have suppressed host immunity defenses. Infants, the elderly, and those who are in the terminal stages of other disease or are immunosuppressed are prime candidates for such infections (Ahmed, 2008).

According to Patricia (2012), *Salmonellosis* causes more disease burden than any other food borne pathogen. An estimated 93.8 million cases of gastroenteritis caused by Salmonella species occur globally each year and of these, nearly 80.3 million cases are food borne (Coker, 2000) In industrialized countries as few as 1% of clinical cases are actually reported (De Champs, 2000) Collectively, Salmonella infections in the United States account for roughly 19,336 hospitalizations, 17,000 quality adjusted life years lost (QALYs), and $3.3 billion in total medical expenditures and lost productivity each year (Mansy, 2001).

Salmonella gastroenteritis is usually a self-limited disease in which the symptom of fever typically resolves within 48 to 72 hours and diarrhoea within three to seven days. Complications from the infection may include severe dehydration, shock, collapse, and/or septicemia. Symptoms are usually more severe among infants, young children, elderly, and those who are immune-compromised (Coker, 2000).

Enterohaemorrhagic E. coli (EHEC)causes bloody diarrhea (hemorrhagic colitis), non-bloody diarrhea and haemolytic uremic syndrome (HUS). The principal reservoir of EHEC is the bovine intestinal tract and initial outbreaks were associated with consumption of undercooked hamburgers. Subsequently, a wide variety of food items have been associated with disease, including sausages, unpasteurized milk, lettuce, cantaloupe melon, apple juice and radish sprouts (Leon, 1950). *Entero toxigenic E. coli* (ETEC) causes watery diarrhea, which can range from mild, self-limiting disease to severe purging disease. The organism is an important cause of childhood diarrhea in the developing world and is the main cause of diarrhoea in travelers to developing countries (Edward, 2010).

Enteroaggregative E. coli (EAEC), are increasingly recognized as a cause of often persistent diarrhea in children and adults in both developing and developed countries, and have been identified as the cause of several outbreaks worldwide (Janda, 2006).

Salvadora persica contain substances that possess plaque inhibiting and antibacterial properties against several types of cariogenic bacteria, which are frequently found in the oral cavity. A comparison study of alcohol and aqueous extract of miswak found that alcoholic extract is more effective than aqueous extract for antibacterial activity. The microorganisms such as *Streptococcus mutans, Lactobacillus acidophilu, Aggregatibacteractinomycetemcomitans, Porphyromonasgingivalis*, and *Haemophilusinfluenzae* were tested against miswak and the results found that the strong antibacterial effects against all bacteria tested is due to the presence of a volatile active antibacterial compounds (Khatak*et al*., 2010). *Also Khatak recommended that Salvadora persica* is a promising product and is useful to produce antiplaque, analgesic, anticonvulsant, antibacterial, antimycotic, cytotoxic, antifertility, deobstruent, carminative, diuretic, astringent, and also used in biliousness, and rheumatism.

The crude extracts of *S. persica* exhibited useful auxiliary antibacterial agent with no toxicity to improve mouth hygiene and treat uncomplicated superficial mouth infections that caused especially by some clinically important bacteria (Al-sieni, 2014). Aqueous extract of *Salvadora persica* (10%) is an effective antimicrobial agent when utilized clinically as an irrigant in the endodontic treatment of teeth with nec-rotic pulps (Al–Salman et al., 2005).

According to Hassan (2011), *S. persica* isa versatile medicinal plant used to treat different human and livestock ailments. It is used for dental care, antiulcer and possesses anti-inflammatory properties. In addition, various parts of *S. persica* are being used as food, fruits and fodder. *S. persica* aqueous extract and methyl alcohol extract were prepared and tested against selected pathogenic microbes: *Staphylococcus aureus, S. mutans, Lactobacillus acifophilus* and *Pseudomonas aeruginosa*, by standard protocol. The aqueous extract showed significant inhibition in the growth of all pathogens tested in the current study. However, S. persica water extract was found to possess profound inhibitory activity against Staphylococcus species as compared to other extracts. Methyl alcohol extract was more active against L. acifophilus and P. aeruginosa. The results indicate promising antibacterial activity of S. persica root extract and recommend further study on its efficacy and safety.

Since *Salvadora persica* has strong antibacterial properties and it is recommending that *S. persica* root can be used to cure disease caused by microorganisms such as *Proteus vulgaris, Escherichia coli, Salmonella typhi, Bacillus cereus and Enterobacter aerogenes.* Further studies are warranted for exploring and identifying the underlying mechanisms of actions of *Salvadora persica* roots on bacteria. Laboratory and clinical investigations of antiviral and antifungal together with the effects of periodontal bacteria need to be performed.

CONCLUSION

The herbaceous extracts of *Salvadora persica* were found to contain glycosides, sterols, terpenes, flavonoids, tannins and alkaloids. The antimicrobial activity of tested medicinal plant can be attributed to any of these constituents. The extract can also be used effectively as a natural tool for teeth cleaning and as a natural analgesic for the disturbing toothache. The phyto medicine is effective in treating most of the infectious disease and is a good alternative to the rural people since it is inexpensive and readily available. It has so many medicinal properties and is a traditional practice so common in large percentage of Maasai community and in India. *Salvadora persica* has strong antibacterial properties. Thus it can be recommended as an important and effective tool for oral hygiene. Most of the secondary metabolites, serve as the plant defends mechanisms against microorganisms, insects and herbivores. A detailed phyto chemical investigation and antimicrobial screening of secondary metabolites from these plant extracts may yield promising antimicrobial agents.

ACKNOWLEDGEMENT

The authors of this paper are very much thankful to the Department of Chemistry and Medical Laboratory Science, University of Eastern Africa, Baraton. The authors also thank the lab assistants for their dedication to ensure the smooth contraction of this study.

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CITE THIS ARTICLE AS:

Anthoney Swamy T and Lasity T Timothy. Phytochemical and Antibacterial Evaluation of Ethanolic extract of *Salvadora persica* Root Extract against Selected microorganisms. *International Journal of Bioassays* 4.12 (2015): 4658-4666.

Source of support: University of Eastern Africa, Baraton, Kenya.
Conflict of interest: None Declared