JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2017. Vol. **11**(3), p. 1337-1349

Phytochemical Screening and Antimicrobial Activities of Stem, Leaves and Fruit Extracts of V*iscum album* L.

Syed Sadaqat Shah[1,2], Yaseen Ur Rehman[2], Arshad Iqbal[2], Zia Ur Rahman[3], Bangwei Zhou[1], Mu Peng[4] and Zhijian Li[1]*

[1]Key Laboratory of Vegetation Ecology, Ministry of Education, Institute of Grassland Science, School of Life Science, Northeast Normal University, Changchun 130024, China.
[2]Department of Botany, Islamia College Peshawar, Peshawar, KPK, Pakistan.
[3]Department of Environmental Sciences, University of Haripur, Pakistan.
[4]College of Life Science, Northeast Forestry University, Harbin 150040, China.

http://dx.doi.org/10.22207/JPAM.11.3.14

(Received: 10 June 2017; accepted: 03 August 2017)

The current research was conducted to study the qualitative analysis and antimicrobial activity of different extracts of *Viscum. album* L. Screening of *Viscum album* for their chemicals was led by familiar qualitative procedures, which exposed the existence of a number of bioactive compounds including alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, reducing sugar and phenols. The antimicrobial activities were investigated by disc diffusion method. Investigation of the data discovered that all of the five extracts of *Viscum album* stems, leaves and fruit showed diverse range of antimicrobial activities. Ethyl acetate, butanol, water and crude extracts showed maximum inhibitory effects against all the microbial species. Especially, Gram-positive bacterial and fungal pathogen. The most liable, gram-positive bacteria were *Bacillus subtilis*, *Bacillus atrophaeus* and *Staphylococcus aureus*, which were repressed by all extracts except n-hexane. The most susceptible gram-negative bacterial species were *Escherichia coli, Erwinia carotovora* and *Agrobacterium tumefaciens* where as the most resilient gram-negative bacterium was *Salmonella typhi.* The results have also supported the practice of aqueous extract were found to be in effect against *Salmonella typhi* and *Escherichia coli*. Thus, our findings have provided support for the use of *Viscum album* stems, leaves and fruit in traditional medicines.

Keywords: Phytochemical screening; antimicrobial activities; *Viscum album*; bacteria; disc diffusion method.

Worldwide the major cause of human death is due to infectious disease (Westh *et al*., 2004). The incidence of food and water contamination has led to a serious health hazard to the community (Aboaba *et al*., 2006). During the latest 50 years, there had been a great deal of concentration in screening plants for beneficial means (Chang *et al*., 2001). The spread of antibiotic resistant pathogens is one of the utmost severe threats to a positive cure of microbial diseases (Prabuseenivasan *et al*., 2006). Although pharmaceutical industries have manufactured a number of new antibiotics in preceding three decades, the resistance to these antibiotics by microbes has increased. Overall, bacteria have chromosomal capability to conduct and attain resistance to medicines, which are consumed as therapeutic agents (Cohen, 1992).

Plant extracts have shown the presence of various chemical constituents such as flavonoids,

* To whom all correspondence should be addressed.
E-mail: khankhanafridi10@yahoo.com

alkaloids, steroids, tannins, saponins, cardiac glycosides and phenol compound, which are synthesized and deposed in specific parts or in all parts of the plants (Parekh *et al*., 2005; Kaur & Arora, 2009). The genus *Viscum* are arboreal hemi-parasites shrubs with twigs 15-80 cm long, which propagates on diverse host trees mainly on Oak and other deciduous trees (Anonymous 1992; Fleming., 1998). Mistletoe has a tendency to form a spherical shape 1 m in diameter. Due to its parasitic nature, the plant is small, dioecious and shrubby. It is dichotomously branching and with diamond shaped olive green rubbery whole leaves and tetra parts flowers, which form white gummy fruits. It has a weak but characteristic odor and unpleasant sense of taste (Bissett, 1994). Worldwide, mistletoe includes 900 species in 65 genera of Loranthaceae family, and 400 species in 7 genera of Viscaceae family (Barlow, 1983).

Earlier research has shown that the leaf of *Viscum album* contains choline, acetylcholine, lectins, polypeptides and polysaccharides. These have presented immune motivating activity in human analyses once mistletoe extracts are given using injection (Hajto, 1986; Bocci, 1993). Other authors also reported some flavanones and chalcones as constituents of *Viscum album*. Cuticle waxes of *Viscum album* show a high content of oleanolic acid with aliphatic constituents such as alkanes, esters, aldehydes, primary alcohols free fatty acid present in much lesser amounts (Wallenweber *et al*., 2002). Previously Oguntoye *et al*., 2008 detected the presence of alkaloids, carbohydrates, tannins and flavonoids. Successive extraction method was monitored for extraction of *V. nepalense* and the consequences discovered the existence of tannins, flavonoids, cardiac glycosides, alkaloids, saponins, reducing compounds and sterols. On the other hand, anthraquinones and triterpenes were lacking (Murali *et al*., 2011). Existence number of viscotoxins, glycosides, alkaloids, phenylpropanoids, tannins, lignins and sugars has been described in the mistletoe collected from diverse host plant life (Orhan *et al*., 2005). The phytochemical side view of mistletoe be influenced by host trees of this plant (Luczkiewicz *et al*., 2001). Mistletoes have been used in the treatment and management of many diseases for many years, both in traditional and complementary medicine (Onay-Ucar et al., 2006). A number of biological effects, such as anticancer, antimycobacterial, antiviral, apoptosis inducing and immuno-modulatory activities have been reported for mistletoes (Onay-Ucar et al., 2006).

The objective of the present study is to identify the existence of phytochemical compounds among Viscum. album L. organs and to test the extracts obtained with difference methods antimicrobial activity against some human pathogenic microbial strains, to conclude some useful anti-microbial principles in future to cope with the emerging need of antimicrobial drugs against these strains.

MATERIALS AND METHODS

Plant material

Viscum album was collected in the month of April from the hills of Khee Kada (Starsapan), Gulistan fort (Manzai Ghar) and Samana hills of Orakzai Agency(Pakistan), located between 33° -33' to 33° -54' north latitudes and 70° -36' to 71° -22' east longitudes. The stems, leaves and fruits of this plant were washed with purified water to get rid of the dust and dirt particle. The plant was dried out in dark at room temperature for two weeks. The entirely dried out stems, leaves and fruits were grinded with electric grinder. The weight of powdered stems, leaves and fruits were measured through electric balance.

Crude extract preparation

For the preparation of crude extract 500g of grinded materials of *Viscum album* stem, leaves and fruit was sinked in methanol taken in round bottom flask for about 24 hours. Then the extracts in solvent form filtered (by watt's man filter paper) to a new round bottom flask. The process of filtration was recurring 3 days using supplementary concentration of methanol (300 ml, 200 ml and 100 ml) earlier the filtration process. The crude (dried out) plant extracts was achieved when evaporation of water via water bath done.

Fractionation of crude extract

10g of crude plant extract was kept back inside round bottom flask. Different concentration of distilled water (200 ml, 150 ml and 100 ml) was poured to the extract contained in flask for three times. Then through separating funnel filtration was done. For concentration of water extract, process of rotary evaporation was performed under

reduced temperature between 30 to 50°C. Similar procedures were adopted for butanol, ethyl acetate and n-hexane.

Phytochemical Screening

Methanol extract were subjected to find the phytochemicals such as alkaloids, saponins, flavonoids, phenol, glycosides, terpenoids, steroids, reducing sugar, tannin, emodin, fatty acid, anthocyanin, coumarin, starch and protein by using standard procedures.

Alkaloids

Take 0.5g of the methanolic extract on filter paper and add some drops of Dragendroff's reagent (solution of potassium Bismuth Iodide). The sample was then detected for the occurrence of yellow precipitation (Tyler, 1994, Harborne et al., 1973a).

Saponins

2 ml of water was added to 2 ml of plant extract in a test tube. Shake the test tube well and wait for frothing for the detection of saponins (Tyler, 1994, Harborne et al., 1973a).

Flavonoids

Take 4 ml of extract and add 1.5 ml of methanol. Warm the mixture besides adding metallic magnesium at that time add 4-5 droplets of hydrochloric acid and proverb the coloration (red) (Tyler, 1994, Harborne et al., 1973b).

Phenoles

To the plant extract 2ml ethanol and few drops of ferric chloride solution were added and observed the coloration. Formation of bluish black color indicates the presence of phenols. (Tyler, 1994, Harborne et al., 1973b).

Glycoside

Add few drops of ferric chloride and concentrated sulfuric acid to the solution of the extract (in glacial acetic acid), and watch for reddish brown coloration at the junction of two layers and the bluish green color in the upper layer (Siddique and Ali., 1997).

Terpenoids and steroid

Take 0.2g of extract and treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then the concentrated sulfuric acid was added slowly. Red violet color denote terpenoids and green bluish color indicates steroids (Siddique and Ali, 1997).

Tannins

To 0.5 ml of methanol extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and green black for catecholic tannins (Iyengar, 1995).

Reducing sugar

Add 1 ml of water and 5-8 drops of Fehling's solution to 0.5 ml of methanolic extract solution and boiled for few minutes. The result was detected for brick red precipitate (Siddique and Ali, 1997).

Coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous extract. Formation of yellow color indicates the presence of coumarins (Rizk, 1982).

Emodins

2 ml of NH4OH and 3 ml of Benzene was added to the extract. Appearance of red color indicates the presence of emodins (Rizk, 1982).

Fatty acid

0.5 ml of extract was mixed with 5 ml of ether. The solution was allowed for evaporation on filter paper and dried. The appearance of transparency on filter paper indicates the presence of fatty acids (Ayoola *et al*., 2008).

Iodine test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicates the presence of the carbohydrate (Harborne, 1973c).

Detection of proteins (xanthoproteic test)

The plant extract was treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins (Prashant *et al*., 2011).

Microorganism used

Gram +ve (*Bacillus atrophaeus*, *Bacillus subtilis* and *Staphylococcus aureus*) and Gram –ve (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Erwinia carotovora*, *Agrobacterium tumefaciens* and *Klebsiella pneumoniae*) bacteria and fungus (*Candida albicans*) were obtain from PCSIR Lab Complex Peshawar, KPK, Pakistan.

Preparation of media

The required quantity of nutrient agar (2.8 g) in 300 ml of purified water and 1.3 g nutrient broth was set in 200 ml purified water. Then it was transferred into cone shaped flask. Approximately 20 ml nutrient broth per test tube was also transferred into test tubes. The entire

media flask and test tube were persevered with cotton wool. For sterilization, these flask and test tube were subjected to maximum temperature (121°C) at 1.5 pound pressure for half an hour (an autoclave). After the sterilization, media (nutrient agar) was tipped into disinfected petri plates in a laminar flow hood to escape any infection. The agar media was permitted to get hard in platters intended for almost 60 minutes. Then the platters were sited in upset position (to evade vaporisation of H_2O from the media) in an incubator at 37 °C for 24 hours. Nearby 24 hours germ-free plates were used for growth of bacterial strain and fungus.

Antimicrobial Activity

Anti-activities of herbal extract were assessed via disc diffusion technique. Subsequently 24 h, bacterial culture was adjusted to 0.5 McFarland turbidity standards. Then these cultures were inoculated on top of nutrient agar plates. Sterilised filter paper disc soaked per plant parts extract in application of 1 and 2 mg disc in 6 and 12 µl volumes was smeared on the disc. Bacterial culture and fungus were incubated at 37 °C for 18 h (Perakh and Chanda 2007).

Positive control

Intended for gram +ve bacteria, Azithromycin 50 µg per6 µl.

Planned for gram –ve bacteria, Levofloxacin 30µg per 6µl.

For *Candida albicans*, Clotrimazole 50 µg per 6µl.

Statistical Analysis

All the data were analysed using the SPSS v. 16 statistical package (SPSS Inc., Chicago, IL, USA). Mean separation of antimicrobial activities of difference organ extracts against pathogenic microorganisms was done with a Tukey's HSD test ($P<0 \cdot 05$).

RESULTS

Phytochemical screening

Phytochemical screening of *Viscum album* for their chemicals was led by familiar qualitative procedures which exposed the existence of a number of bioactive compounds including alkaloids, saponins, tannins, glycosides, flavonoids, terpenoids, steroids, emodin, reducing sugar, coumarin, phenols and proteins; however, the fatty acid, anthocyanin and starch were not found in all the plant parts (Table 1).

Antimicrobial activities

Table 2 shows the antimicrobial activities of stem extracts of *Viscum album* against different human pathogenic microorganisms by disc diffusion method. Analysis of the data revealed that *B. atrophaeus* was susceptible to all the extracts except n-hexane. Against *B. atrophaeus* the highest zone of inhibition was shown by methanol extract. Which was showed 35.00 mm ZI at both the concentrations. In case of *B. subtilis,* butanol extracted samples at both concentrations and methanol extracted sample at high concentration showed the same effect as positive control, while no inhibition was observed for n-hexane extract. *S. aureus* was susceptible to all the extracted samples of the stem of *Viscum album*. The best zone of inhibition was observed by methanolic extract, which showed 35.33 and 35.66 mm ZI at 1 and 2 mg $disc^{-1}$, respectively. Aqueous and butanol extracted samples were also effective against *S. aureus* and showed good ZI. While n-hexane and Ethyl acetate were moderately effective against *S. aureus*. *P. aeruginosa* was sensitive to Ethyl acetate, butanol, methanol and aqueous extracts at both the concentrations used, however n-hexane extract showed no inhibitory effect on *P. aeruginosa*, measuring no zone of inhibition. Our results also suggested that *E. coli* were the most susceptible to the Ethyl acetate extract as compared to other extracts and showed 20.33 and 23.33 ZI at 1 and 2 mg $disc^{-1}$, respectively. However, no ZI was observed against *E. coli* while using n-hexane extract of stem of *V. album*. Against the *S. typhi* all the extracts showed ZI at both the concentrations except n-hexane extract. *S. typhi* was more sensitive to the Ethyl acetate extract as compared to other extracts and showed 20.66 and 25.33 ZI at 1 and 2 mg $disc^{-1}$, respectively. The data revealed that *E. carotovora* were completely resistant to the n-hexane extract. However the same bacterium was sensitive to Ethyl acetate, butanol, methanol and aqueous extracted samples of stem of *V. album*. Highest zone of inhibition was recorded by Ethyl acetate extract which was 25.00 and 27.00 mm ZI at 1 and 2 mg $disc^{-1}$, respectively. While butanol extract at higher concentrations showed the same effect as positive control. *A. tumefaciens* were sensitive to all the extracts at both the concentrations. The lowest ZI was recorded by aqueous extract while n-hexane,

Table 1. Results of phytochemical screening on crude methanol extracts of stems, leaves and fruits of *Viscum album*.

S. No	Compounds	*V. album* Stem Methanol	*V. album* Leaves Methanol	*V. album* Fruit Methanol
1	Saponins	+	+	+
2	Alkaloids	+	+	+
3	Reducing sugar	+	+	+
4	Glycosides	+	+	+
5	Terpenoids	+	+	+
6	Steroids	+	+	+
7	Tannins	+	+	+
8	Flavonoids	+	+	+
9	Emodin	+	+	+
10	Fatty acid	–	–	–
11	Anthocyanin	–	–	–
12	Coumarin	+	+	+
13	Phenols	+	+	+
14	Starch	–	–	–
15	Proteins	+	+	+

+: Represents presence of the phytoconstituent;–: represents absence of the phytoconstituent

Table 2. Antimicrobial activities of stem extracts of *Viscum album* against different human pathogenic microorganisms by disc diffusion method. Values are the mean of 3 replications for stem extracts by using disc diffusion method. Means followed by different letters are significantly different ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test.

Organisms	Conc. mg disc^{-1}	Mean diameter of zones of inhibition (mm)					
		n-Hexane extract	Ethyl acetate extract	Butanol extract	Methanol extract	Aqueous extract	Positive Control
B. atrophaeus	1	-	22.00^{d}	25.00^{c}	35.00^{a}	30.66^{b}	30.33^{b}
	2	-	27.33^{c}	25.00^{d}	35.00^{a}	30.00^{b}	33.66^{a}
B. subtilis	1	-	20.66^{c}	30.33^{a}	26.00^{b}	25.00^{b}	30.00^{a}
	2	-	25.00c	33.33^{a}	34.66^{a}	30.33^{b}	34.33^{a}
S. aureus	1	20.00^{c}	20.66^{c}	30.00^{b}	35.33^{a}	30.33^{b}	30.33^{b}
	2	22.00^{e}	27.66^{d}	30.00^{c}	35.66^{a}	30.66^{b}	32.33^{b}
P. aeruginosa	1	-	20.00^{c}	20.33^{c}	22.33^{b}	20.00^{c}	30.66^{a}
	2	-	24.00^{c}	23.66^{c}	20.33^{d}	28.33^{b}	31.00^{a}
E. coli	1	-	20.33^{b}	15.66^{c}	15.66^{c}	15.33^{c}	32.00^{a}
	2	-	24.33^{b}	20.33^{c}	20.33^{c}	18.33^{d}	32.33^{a}
S. typhi	1	-	20.66^{b}	20.00^{b}	18.00^{c}	15.66^{d}	30.00^{a}
	2	-	25.33^{b}	23.00^{c}	20.00^{d}	20.00^{d}	36.00^{a}
E. carotovora	1	-	25.00^{a}	22.33^{b}	20.33^{c}	15.00^{d}	25.66^{a}
	2	-	27.00^{a}	25.66^{a}	20.66^{b}	20.00^{b}	27.33^{a}
A. tumefaciens	1	20.33^{b}	20.33^{b}	20.66^{b}	20.00^{b}	15.00^{c}	30.00^{a}
	2	25.66^{b}	25.00^{b}	25.66^{b}	22.00^{c}	20.00^{c}	32.00^{a}
K. pneumoniae	1	-	25.33^{b}	19.33^{d}	22.33^{c}	13.66^{e}	30.33^{a}
	2	-	27.66^{b}	19.33^{d}	27.00^{b}	22.66^{c}	36.00^{a}
C. albicans	1	13.33e	17.00^{d}	25.66^{b}	20.66^{c}	12.00^{e}	33.00^{a}
	2	20.66^{d}	20.33^{d}	30.00^{b}	25.00^{e}	17.00^{e}	33.66^{a}

Ethyl acetate, butanol and methanol extract showed good activity against *A. tumefaciens*. Our results also showed that *K. pneumoniae* were the most susceptible bacterium to Ethyl acetate extract and the same bacterium were resistant to n-hexane extract of stem of *V. album*. While moderate ZI was also observed by other extracts. *C. albicans* were sensitive to all the extracted samples at both the concentrations while best ZI was observed by Butanol extract, which showed 25.66 and 30.00 mm ZI at 1 and 2 mg disc^{-1}.

Data regarding the antibacterial activity of leaves extracts of *Viscum album* against different human pathogenic microorganisms by using disc diffusion method is shown in the Table 3. The data indicated that *B. atrophaeus* was highly susceptible to the butanol and methanol extracts of the leaves of the *V. album*. All the extracts showed good reduction in the growth of *B. atrophaeus*. But among these extracts butanol and methanol extracts reduced the growth of *B. atrophaeus* and showed 30.00 and 30.66 mm ZI at the concentration of 1 mg disc^{-1}, respectively. However, n-hexane extract didn't show any activity against *B. atrophaeus*. All the extracts showed good to moderate antibacterial activity against *B. subtilis* except n-hexane. Which didn't inhibit the *B. subtilis* growth at any concentration. Among these extracts, butanol extract showed highest zone of Inhibition (24.66 and 30.00 mm 1 and 2 mg disc^{-1}) when compared to the other extracts. Our results also indicated that all the extracts showed good antibacterial activity against *S. aureus*. But among these extracts methanol extract had a profound inhibitory activity against *S. aureus*. Which showed 30.66 ZI at both the concentrations (1 and 2 mg disc^{-1}), respectively. While Ethyl acetate extract at higher concentration showed the same effect as positive control. *P. aeruginosa* was resistant to both n-hexane and aqueous extracts and didn't inhibit the growth of *P. aeruginosa* at any concentration. While other extracts showed good to moderate activity when

Table 3. Antimicrobial activities of leaves extracts of *Viscum album* against different human pathogenic microorganisms by disc diffusion method. Values are the mean of 3 replications for leaves extracts by using disc diffusion method. Means followed by different letters are significantly different ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test.

Organisms	Conc. mg disc^{-1}	Mean diameter of zones of inhibition (mm)					
		n-Hexane extract	Ethyl acetate extract	Butanol extract	Methanol extract	Aqueous extract	Positive Control
B. atrophaeus	1	-	22.00^{c}	30.00^{a}	30.66^{a}	24.33^{b}	30.33^{a}
	2	-	25.00^{d}	32.00^{b}	30.66^{b}	30.00^{c}	33.66^{a}
B. subtilis	1	-	22.33^{c}	24.66^{b}	24.00^{b}	13.66^{d}	30.00^{a}
	2	-	25.33^{d}	30.00^{b}	27.00^{c}	20.33^{e}	34.33^{a}
S. aureus	1	15.00^{c}	26.66^{b}	25.33^{b}	30.66^{a}	13.00^{c}	30.33^{a}
	2	25.00^{c}	30.66^{a}	28.66^{b}	30.66^{a}	20.33^{d}	32.33^{a}
P. aeruginosa	1	-	20.00^{c}	20.00^{c}	25.33^{b}	-	30.66^{a}
	2	-	22.00^{d}	24.00^{c}	25.66^{b}	-	31.00^{a}
E. coli	1	-	17.33^{c}	20.33^{b}	20.00^{b}	-	32.00^{a}
	2	-	20.66^{b}	20.66^{b}	20.00^{b}	-	32.33^{a}
S. typhi	1	-	17.00^{c}	22.66^{b}	22.00^{b}	12.33^{d}	30.00^{a}
	2	-	20.33^{d}	22.66^{c}	30.00^{b}	15.33^{e}	36.00^{a}
E. carotovora	1	-	19.00^{c}	25.33^{b}	30.66^{a}	13.00^{d}	25.66^{b}
	2	-	25.66^{b}	26.66^{b}	30.66^{a}	15.00^{c}	27.33^{b}
A. tumefaciens	1	16.33^{c}	19.33^{b}	15.00^{c}	19.33^{b}	15.66^{c}	30.00^{a}
	2	16.66^{c}	25.00^{b}	22.00^{b}	23.66^{b}	18.66^{c}	32.00^{a}
K. pneumoniae	1	-	22.00^{b}	20.33^{b}	22.00^{b}	16.33^{c}	30.33^{a}
	2	-	25.00^{b}	25.66^{b}	22.33^{c}	20.66^{c}	36.00^{a}
C. albicans	1	12.00^{e}	18.33^{d}	20.66^{c}	25.00^{b}	12.66^{e}	33.00^{a}
	2	18.33^{c}	20.66^{c}	30.66^{b}	30.66^{b}	12.66^{d}	33.66^{a}

compared to standard antibiotics. Among them, methanol extract showed 25.33 and 25.66 ZI at both 1 and 2 mg disc^{-1}, respectively. *E. coli* was also resistant to both the n-hexane and aqueous extract of leaves of *V. album* and didn't show any ZI at any concentration. While butanol and methanol extract showed good activity when compared to the other extracts and standard. Both the butanol and methanol extracted samples showed good activity at both the concentrations, while Ethyl acetate extract showed highest inhibition of 20.66 mm at 2 mg disc^{-1}, when compared to the antibiotics. Our data also indicated that *S. typhi* was completely resistant to n-hexane extract and didn't show any ZI. The best activity was observed by methanol extract which reduced the growth of *S. typhi* as 22.00 and 30.00 at 1 and 2 mg disc^{-1}, when compared to other extracts. Similarly, *E. carotovora* was also completely resistant to n-hexane extract and showed no activity. While the best activity was observed by the methanol extract which showed 30.66 mm ZI at both the concentrations. Methanol extract of leaves of *V. album* reduced the growth of *E. carotovora* more than the standard antibiotic. *A. tumefaciens* were susceptible to all the extracts and showed good to moderate antibacterial activity when compared to the standard antibiotics. Among these extracts, *A. tumefaciens* were more susceptible to the Ethyl acetate and methanol extracts and showed 25.00 and 23.66 mm ZI at 2 mg disc^{-1}, respectively. Our data also reveal that n-hexane extract were ineffective to control the growth of *K. pneumoniae* and didn't show any ZI. While Ethyl acetate, butanol, methanol and aqueous were active to control the growth of *K. pneumoniae*. Among these extracts, Ethyl acetate and butanol were more active to control the growth of *K. pneumoniae* and showed 25.00 and 25.66 mm ZI at 2 mg disc^{-1}. Our results also indicated that n-hexane, Ethyl acetate, butanol, methanol and aqueous all were effective against *C. albicans* at both the concentrations.

Table 4. Antimicrobial activities of fruit extracts of *Viscum album* against different human pathogenic microorganisms by disc diffusion method. Values are the mean of 3 replications for fruit extracts by using disc diffusion method. Means followed by different letters are significantly different ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test

Organisms	Conc. mg disc^{-1}	Mean diameter of zones of inhibition (mm)					
		n-Hexane extract	Ethyl acetate extract	Butanol extract	Methanol extract	Aqueous extract	Positive Control
B. atrophaeus	1	20.33^{d}	28.33^{b}	25.00^{c}	30.33^{a}	15.66^{e}	30.33^{a}
	2	25.66^{c}	30.66^{b}	30.33^{b}	35.33^{a}	22.33^{d}	33.66^{a}
B. subtilis	1	20.00^{c}	25.00^{b}	30.00^{a}	30.00^{a}	15.00^{d}	30.00^{a}
	2	22.00^{d}	26.66^{c}	30.00^{b}	34.00^{a}	22.66^{d}	34.33^{a}
S. aureus	1	10.66^{d}	25.66^{b}	30.33^{a}	25.33^{b}	17.33^{c}	30.33^{a}
	2	15.66^{d}	30.66^{b}	32.66^{a}	35.00^{a}	20.66^{c}	32.33^{b}
P. aeruginosa	1	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	10.00^{c}	31.66^{a}
	2	20.00^{c}	25.00^{b}	21.00^{c}	25.00^{b}	15.00^{d}	31.00^{a}
E. coli	1	17.33^{c}	18.33^{c}	-	20.33^{b}	13.33^{d}	32.00^{a}
	2	20.33^{b}	20.66^{b}	-	22.33^{b}	14.66^{c}	32.33^{a}
S. typhi	1	-	-	16.66^{c}	22.00^{b}	13.00^{d}	30.00^{a}
	2	-	-	20.66^{c}	25.00^{b}	13.33^{d}	36.00^{a}
E. carotovora	1	-	22.33^{b}	23.00^{b}	26.33^{a}	13.33^{c}	25.66^{a}
	2	-	25.66^{c}	23.00^{d}	30.33^{a}	13.00^{e}	27.33^{b}
A. tumefaciens	1	-	15.66^{c}	15.33^{c}	22.66^{b}	10.33^{d}	30.00^{a}
	2	-	20.00^{c}	20.00^{c}	25.66^{b}	15.00^{d}	32.00^{a}
K. pneumoniae	1	20.33^{c}	22.33^{c}	19.33^{d}	25.33^{b}	17.66^{d}	30.33^{a}
	2	20.66^{c}	24.66^{b}	20.66^{c}	26.33^{b}	20.00^{c}	36.00^{a}
C. albicans	1	22.00^{b}	17.33^{c}	15.00^{d}	20.33^{b}	10.00^{e}	33.00^{a}
	2	22.00^{b}	20.33^{b}	20.00^{c}	22.33^{b}	14.00^{d}	33.66^{a}

Among these extracts methanol was more effective to reduce the growth of *C. albicans*. Which reduced the growth of *C. albicans* as 25.00 and 30.00 mm ZI at 1 and 2 mg disc^{-1}, respectively.

The antimicrobial activity of n-hexane, ethyl acetate, butanol, methanol and aqueous extracts of fruit of *Viscum album* is shown in Table 4. Our results reveal that all extracts were effective in reducing the growth of *B. atrophaeus*. Methanol extract showed tremendous inhibition of 30.33 at 1 mg disc^{-1} and 35.33 mm at concentration of 2 mg disc^{-1} as compared with antibiotics. Butanol and Ethyl acetate also showed good activity as compared to n-hexane and aqueous extracts. Similarly, *B. subtilis* were inhibited by all the extracts of *V. album*, but methanol had a profound inhibitory effect against *B. subtilis*, which reduced the growth of *B. subtilis* as 30.00 and 34.00 mm ZI at 1 and 2 mg disc^{-1}, respectively. While butanol extract showed 30.00 mm ZI against *B. subtilis* at both the concentrations. The effect of butanol extract at lower concentration was the same as the positive control. Against *S. aureus* butanol showed tremendous inhibition of 30.33 mm ZI at 1 mg disc^{-1} and 32.66 mm ZI at 2 mg disc^{-1}, respectively. While methanol extract at higher concentration showed the same effect as positive control. As compared to the other extracts n-hexane showed the lowest inhibition against *S. aureus*. Our data also suggested that *P. aeruginosa* was completely susceptible to all the fruit extracts of *V. album* and showed good to moderate activity when compared to positive control. Among all the extracts, Ethyl acetate and methanol extracts showed 20.00 and 25.00 mm ZI at both 1 and 2 mg disc^{-1}, respectively. *E. coli* was completely resistant to butanol extract of fruit of *V. album* and didn't show any zone of inhibition. While all the other extracts were effective in controlling the growth of *E. coli*. Among them methanol extract showed the best activity when compare to the positive control. Which reduced the growth of *E. coli* as 20.33 mm ZI at 1 mg disc^{-1} and 22.33 mm ZI at 2 mg disc^{-1}, respectively. N-hexane extract and ethyl acetate extract, both were unable to control the growth of *S. typhi*, which didn't show any inhibitory activity at any concentration. While among the other extracts methanol extract showed good activity and reduced the growth of *S. typhi* as 22.00 and 25.00 mm ZI at the concentrations of 1 and 2 mg disc^{-1}, respectively. Our results also indicated that all the extracts were active against *E. carotovora* except n-hexane, which didn't inhibit the *E. carotovora* at any concentration. Methanol extract showed tremendous antibacterial activity more than standard antibiotic against *E. carotovora* and showed 26.33 and 30.33 mm ZI at the concentration of 1 and 2 mg disc^{-1}, respectively. N-hexane didn't show any effectiveness against *A. tumefaciens* at any concentration while other extracts showed good to moderate activity when compared to positive control. Among the extracts, methanol extract showed the best inhibition of 22.66 mm ZI at 1 mg disc^{-1} and 25.66 mm ZI at 2 mg disc^{-1}, respectively. N-hexane, Ethyl acetate, butanol, methanol, aqueous extracts all were effective against *K. pneumoniae* and inhibited the *K. pneumoniae* at both the concentrations. The highest inhibition was recorded by methanol extract which were 25.33 and 26.33 mm ZI at both 1 and 2 mg disc^{-1}, as compared to other extracts. Our data also reveal that n-hexane extract of fruit of *V. album* showed the best activity against *C. albicans* and reduced the growth of *C. albicans* better than other extracts. The growth which were reduced by the n-hexane extract was 22.00 mm ZI at both the concentrations (1 and 2 mg disc^{-1}).

DISCUSSION

Various chemical compounds such as alkaloids, flavonoids, glycosides, phenol, saponins and sterols etc. are present in medicinal plants responsible for the improvement of human health. The primary screening experiments are helpful in the discovery of the chemical constituents, which can bring about the drugs isolation and advancement (Mallikharjuna *et al.,* 2007). The phytochemical analysis conducted on *Viscum album* methanolic extract revealed the presence of alkaloids, saponins, tannins, glycosides, flavonoids, terpenoids, steroids, emodin, reducing sugar, coumarin, phenols and proteins. These results agree with (Umoh *et al*., 2011; Ihegboro and Ebuehi, 2012). However, Oguntoye *et al*., (2008) did not observed saponins and terpenoids in the ethanolic and aqueous extract of *Viscum album* plant.

In the present study investigates the antimicrobial activities of different solvent

extracted samples form the stem, leaves and fruits of *V. album* by using disc diffusion method. Ten bacterial species were used, in which six were gram negative and 3 were gram positive and one fungus (*C. albicans*). Our results reveal that *B. atrophaeus* was susceptible to the Ethyl acetate, butanol, methanol and aqueous extracts of the stem of *V. album*. Among them *B. atrophaeus* was the most sensitive to the methanol extract and showed highest inhibition as compared to other extracts and positive control. N-hexane extract of stem of *V. album* did not show any inhibition against *B. atrophaeus*. Hussain et al., (2011) observed 20.06 and 15.03 ZI against gram positive bacteria (*E.* faecium) while using ethyl acetate and methanol extract of twigs of *V. album*. B. *subtilis* were sensitive to all the extracts of stem of *V. album* except n-hexane extract. The highest zone of inhibition was recorded by butanol extract, which was better than positive control and other extracts. Hussain *et al*., (2011) used different extracts of twigs of *V. album* against different bacteria. He used methanol and Ethyl acetate extracts of twigs of *V. album* and observed observe 19.9 mm and 15.06 mm ZI against *B. subtilis* while using the concentration of 100mg/ml. Our results also reveal that *S. aureus* was sensitive to all the extracts of stem of *V. album* and showed inhibition at both the concentrations. Methanol extract showed tremendous inhibition against the *S. aureus* as compared to other extracts and positive control. Similar results were also reported by Hussain et al., (2011). Our results also reveal that *P. aeruginosa* was completely resistant to n-hexane extract of stem of *V. album*, while other extracts showed good to moderate effect against *P. aeruginosa*. Hussain et al., (2011) observed 19.66 and 17.03 mm ZI against *P. aeruginosa*, while using the Ethyl acetate and methanol extract of twigs of V. album, at concentration of 100mg/ml. Our results suggested that *E. coli* was sensitive to all the extracts except N-hexane extract. The maximum inhibition was recorded by Ethyl acetate extract as compared to other extracts. While butanol and methanol extracts showed the same inhibition against *E. coli* at both the concentrations. Similarly, Hussain *et al*., (2011) used different extracts of twigs of V. album. He used Ethyl acetate and methanol extract of V. album against E. coli and recorded 20.83 mm ZI by both extracts. Our results also reveal that *S. typhi* was completely resistant to n-hexane extract of stem of *V. album* and didn't show any zone of inhibition at any concentration. While Ethyl acetate extract showed good zone of inhibition as compared to the other extracts. Similar result was also reported by Hussain *et al*., (2011). Against *E. carotovora*, Ethyl acetate extract showed highest inhibition as compared to positive control and other extracts. The effect of Ethyl acetate extract of stem of *V. album* against *E. carotovora* was similar to as positive control. While our results also reveal that *E. carotovora* was completely resistant to n-hexane extract of stem of *V. album*. *A. tumefaciens* was sensitive to all the extracts and showed zone of inhibition at both the concentrations. The effect of n-hexane, Ethyl acetate and butanol extract were the same when compared to methanol and aqueous. The lowest zone of inhibition was observed by the aqueous extract. *K. pneumoniae* was completely resistant to the n-hexane extract of stem of *V. album*, while other extracts showed good to moderate activity against *K. pneumoniae*. Among them Ethyl acetate extract recorded the highest zone of inhibition against *K. pneumoniae* when compared to the other extracts. Our results also reveal that all the extracts were active against the *C. albicans* and showed zone of inhibition at both the concentrations. The maximum zone of inhibition was recorded by butanol extract when compared to the other extracts.

Our results further reveal that *B. atrophaeus* was susceptible to the Ethyl acetate, butanol, methanol and aqueous extracts of the leaves of *V. album*. While same bacteria was completely resistant to n-hexane extract of leaves of *V. album*. The highest zone of inhibition was recorded by the methanol and butanol extract at 1 mg $disc^{-1}$, when compared to the other extracts. Yusuf et al., (2013) used different extracts of leaves of V. album against different human pathogen. He observed 12 and 8 mm ZI against *B. cereus*, while using methanolic and hexane extract at concentration of 30mg/ml. *B. subtilis* was found completely resistant to the n-hexane extract and didn't stop the growth of *B. subtilis* at any concentration. While same bacteria was inhibited by the other extracts of leaves of *V. album*. The highest zone of inhibition was recorded by the butanol extract as compared to the other extracts. Hussain et al., (2011) used five extracts of leaves

of V. album against different human pathogens. He observed 24.33, 15.16, 15.9 and 9.66 mm ZI against B. subtilis, while using the Ethyl acetate, methanol, ethanol and aqueous extract. He did not observe any inhibition against B. subtilis while using the chloroform extract of leaves of V. album. *S. aureus* was sensitive to all the extracts of the leaves of the *V. album* and showed inhibition by both the concentrations. Among all the extracts, the highest zone of inhibition was recorded by methanol extract as compared to the positive control and other extracts. Methanol extract reduced the growth of *S. aureus* better than positive control. Yusuf *et al*., (2013) observed 13.33 mm ZI against S. aureus, while using the methanol extract of leaves of *V. album* at the concentration of 30mg/ml. our results were contrary to Oguntoye *et al*., (2008), because he did not observe any inhibition against S. aureus while using the aqueous extract of leaves of *V. album*. Our results are also not in agreement with Hussain *et al*., (2011), because he did not record highest inhibition by using methanol extract. He observed 24.33 mm ZI by using Ethyl actate extract and 15.16 mm ZI by using methanol extract. *P. aeruginosa* was completely resistant to n-hexane extract and aqueous extract and showed no zone of inhibition at any concentration. Ethyl acetate, butanol and methanol was found active against *P. aeruginosa* and inhibit the bacteria at both the concentrations. Methanol showed the best activity among other extracts. Similar results were also reported by Hussain et al., (2011). Our results are not parallel to Oguntoye et al., (2008), because he observed 9.00 mm ZI against P. aeruginosa by using aqueous extract of leaves of V. album. Our results also demonstrated that n-hexane and aqueous extracts did not reduced the growth of *E.coli* at any concentrations, however, Ethyl acetate, butanol and methanol inhibit its growth. Highest reduction was noted by the butanol and methanol extracts. Our results are not in agreement with Yusuf et al., (2013) because he observe 9 mm ZI against E. coli by using aqueous extract of V. album. Our results are also not parallel to the Hussain et al., (2011), because he observed highest inhibition by Ethyl acetate extract. He observed 24.96, 16.93 and 9.16 mm ZI against E. coli by using Ethyl acetate, methanol and aqueous extract at concentration of 100 mg/ml. All the extracts show antimicrobial activity against *S. typhi* except n-hexane extract of leaves of *V. album*. Among these extracts, methanol extract show good inhibition against *S. typhi*. . Similar results were also reported by Yusuf et al., (2013). Our results are not parallel with Hussain et al., (2011), because he did not observe any ZI against S. typhi by using Ethyl acetate extract, while he observes 15.26 and 9 mm ZI by using methanol extract and aqueous extract of leaves of V. album. Our results also suggested that the growth of E. carotovora was reduced by the Ethyl acetate extract, butanol, methanol and aqueous extracted samples of leaves of *V. album*. N-hexane extract did not show any activity against *E. carotovora*. Among these extracts, methanol extract showed tremendous activity and reduced the growth of *E. carotovora* better than positive control and other extracts. Our results further indicated that all the extracts were effective against *A. tumefaciens*. The highest zone of inhibition was recorded by Ethyl acetate extract and methanol extract at both the concentrations. *K. pneumoniae* was completely resistant to the n-hexane extract of leaves of *V. album*. While all other extracts were active against *K. pneumoniae*. Among these extracts, highest zone of inhibition was recorded by the Ethyl acetate extract and butanol extract at both the concentrations. Our results are not in agreement with Yusuf et al., (2013), because he observed 4, 5 and 6.33 mm ZI against k. pneumoniae by using hexane, ethanolic and methanolic extract at the concentration of 30 mg/ml. Similarly, Oguntoye *et al*., (2008), observed 12.00 mm ZI against K. pneumoniae by using aqueous extract of leaves of *V.album*. Our results also demonstrated that *C. albicans* was sensitive to all the extracts of leaves of *V. album* and show inhibition at both the concentrations. Highest zone of inhibition was recorded by methanol extract.

Our results revealed that all the extracted samples of fruits of *V. album* were effective to control *B. atrophaeus* at both the concentrations. The highest zone of inhibition was recorded by methanolic extract followed by Ethyl acetate extract. Methanol extract reduced the growth better than positive control and other extracts. *B. subtilis* was susceptible to n-hexane extract, Ethyl acetate, butanol, methanol and aqueous extracts of leaves of *V. album*. The best zone of inhibition was recorded by methanol extract follow by butanol extract. In case of *S. aureus*, all the extracts show effectiveness

and inhibit the bacteria at both concentrations. Butanol had a profound inhibitory activity against *S. aureus* and inhibit it at both the concentrations. N-hexane, Ethyl acetate, butanol, methanol and aqueous extracts all were effective against *P. aeruginosa* and recorded zone of inhibition at both the concentrations. The best zone of inhibition was recorded by methanol extract and butanol extracts at both the concentrations. Our results also demonstrated that *E. coli* was completely resistant to the butanol extract of fruits of *V. album*, while all other extracts inhibit the *E. coli* at both the concentrations. *E. coli* was the most sensitive to the methanol extracted sample and recorded best zone of inhibition at both the concentrations among all the extracts. Our results also suggested that *S. typhi* show resistance to both n-hexane extract and Ethyl acetate extract and did not recorded zone of inhibition at any concentration. While among the other extracts methanol show good activity against *S. typhi* and recorded the highest zone of inhibition at both the concentrations. Our results also suggested that methanol extract of fruits of *V. album* had a tremendous antibacterial activity against *E. carotovora*. The zone of inhibition was recorded against *E. carotovora* by methanol extract was better than positive control and other extracts. While the same bacteria was completely resistant to n-hexane extract. Our data also indicated that Ethyl acetate, butanol, methanol and aqueous extracts were found effective against *A. tumefaciens* while n-hexane extract did not inhibit *A. tumefaciens* at any concentration. The highest zone of inhibition was recorded by methanol extract followed by Ethyl acetate extract and butanol extract. Our results also demonstrated that *K. pneumoniae* were susceptible to all the extracts of fruits of *V. album*. The highest zone of inhibition was recorded by methanol extract at both the concentrations. While against *C. albicans* all the extracts were effective and show zone of inhibition at both the concentrations. The highest ZI was recorded by n-hexane extract and methanol extract.

The use of hexane, ethyl acetate, butanol, water and crude (methanol) as take out solvents verified to be more capable in extracting the active compounds. The antibacterial activities of the hexane, ethyl acetate, butanol, water and methanol extracts were match to Azithromycin, Levofloxacin and Clotrimizole (standered antibiotics). Independent of gram reaction, these antibiotics gives the impression to be wide range in its activities. The antibiotic (Clotrimazol) were use against C. albicans also given positive results. The tested plant parts revealed the maximum activity against certain bacteria than the antibiotics used, representing that this plant is good source of antibiotics for the cure of certain bacteriological illnesses. However, further experimental and research determinations on this plant and their extracts are necessary to be able to state the pharmacological suggestion. Other details requirement include tests other solvents, infrared spectrometry, (mass spectrometry) MS and nuclear magnetic resonance (NMR) of the constituents of the extracts.

The biological activities of the plant tested can help to discover new antibiotics, which can be use as selective agents for controlling infectious diseases. The results indicated the possibility that stems, leaves and fruits of *Viscum album* can be used for the cure of several types of ailments. Two types of extracts (Ethyl acetate and crude) have presented good action in contrast to different bacteria and fungi. The mentioned extracts would be checked for advance studies. It is also suggested to separate the bioactive compounds of *Viscum album* stems, leaves and fruits and then to practice the activity of each individual compound against diverse microbial strains.

CONCLUSIONS

It is concluded from the result that stems, leaves and fruits of *Viscum album* is composed of comparatively great amount of phytochemicals such as alkaloids, flavonoids, saponins, steroids, glycosides and phenols etc. These antimicrobial bioactive components mark the tested plant a confident candidate for future research and for medicinal usages.

ACKNOWLEDGMENTS

This work was supported by The National Program on Key Basic Research Project (2015CB150801), Key Science and Technology Program of Jinlin Province (2012ZDGG008), and Jilin Province Science and Technology Development Plan Project (20170414051GH).

REFERENCES

1. Aboaba, O.O., Smith, S.I., Olude, F.O. Antibacterial effect of edible plant extracts of *Escherachia coli* 0157:H7. *Pak. J. Nutr*., 2006; **5**(4): 325-327.
2. Anonymous. The Lawrence Review of Natural products. In: Charles Dombek R, MA, ed. Facts and comparisons. St. Louis: *J. B.*, *Lippencott Company*. 1992.
3. Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C., Atangbayila T.O. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. *Trop. J. Pharm. Res*., 2008; **7**: 1019-1024.
4. Barlow, B.A. Mistletoe in focus: Introduction. In D. M. Calder and P. Bemhardt (Eds) Biology of mistletoe, *AcdemicmPress Inc*. NY., 1983; pp 46.
5. Bissett, N.G. Herbal drugs and phytopharmaceuticals. Stuttgart: *Medpharm CRC Press*: 1994; pp 566.
6. Bocci, B. Mistletoe (*Viscum album*) Lectins as cytokine inducers and Immuno- adjuvant in tumour therapy. *A review. J. Biol. Regulatory Homoestatic Agents*., 1993; **7**:1-6.
7. Chang, J.C., Chiang, L.C., Chen, C.C., Liu, L.T, Wang, K.C., Lin, C.C. Antileukemic activity of *Bidens pilosa* var. minor (Blume) Sherff and *Houttuynia cordata* Thunb. *Ameri. J. Chin. Med.,* 2001; **29**: 303–312.
8. Cohen, M. L. Epidemiology of drug resistance: Implications for a postantimicrobial era. *Science*., 1992; **257**: 1050–1055.
9. Erturk, O., Kati, H., Yayli, N., Demirbag Z. Antimicrobial activity 0f Viscum album L. subsp. abietis (wiesb). *Turk J. Biol*., 2003; **27**:255-258.
10. Fleming, T. PDR for herbal medicines. Montvale, NJ: *Medical Economics Company, Inc.,* 1998;
11. Hajto, J. Immunomodulatory effects of Iscador. A *Viscum Album* preparation. *Oncology*., 1986; **43**:51-63.
12. Harborne, J.B. Phytochemical methods, London Chapman and Hall, Ltd. 1973a; pp. 49-88.
13. Harborne, J.B. Phytochemical methods. Chapman and hall, London. 1973b; Pp. 1.
14. Harborne, J.B. Phytochemicals Methods. *Chapman and Hall Ltd*., *London*. 1973c; pp. 49-188.
15. Hussain, M.A., Khan, M.Q., Hussain, N., Habib, T. Antibacterial and Antifungal potential of leaves and twigs of *Viscum album L. J. Med. Plant. Res*., 2011; **5**(23):5545-5549.
16. Ihegboro, G.O., Ebuehi O.A.T. Phytochemical Investigation and the Effects of Aqueous Plant Extracts of *Viscum Album* on Antioxidant Property and Biochemical Profile as a Measure of its Therapeutic Value. *International Journal of Science and Research (IJSR)*., 2012; **3**(8): 1192-1995.
17. Iyengar, M.A. Study of Crude Drugs. 8th ed., Manipal Power Press, Manipal, India., 1995; pp 2.
18. Kaur, G.J., Arora, D.S. Antibacterial and phytochemical screening of *Anthum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. *Biomed Central complementary and Alternative medicine*., 2009; **9**(30): 1-30.
19. Luczkiewicz, M., Cisowski, W., Kaiser, P., Ochocka, R., Piotrowski A. Comparative analysis of phenolic acids in mistletoe plants fromvarious hosts. *Acta Poloniae Pharmaceutica-Drug Res.,* 2001; **58**(5): 373-379.
20. Mahesh, B., Satish, S. Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. *World J. Agr. Sci*., 2008; **4**(S): 839-843.
21. Mallikharjuna, P.B., Rajjana, L.N., Seetharam, Y.N., Sharanasappa, G.K. Phytochemical studies of *Strychnos potatorum* L. f.- A Medicinal plant. *E-Journal of Chemistry.*, 2007; **4**(4): 510-518.
22. Murali, M., Puneetha, G.K., Thriveni, M.C., Niranjan, M.H., Shivamurthy, G.R., Niranjana, S.R., Prakash, H.S., Amruthesh, K.N. Phytochemical screening and antioxidant activity of hemi-parasitic Indian mistletoe *Viscum nepalense Sprengel. Journal of Pharmacy Research*., 2011; **4**(10):3348-3350.
23. Oguntoye, S.O., Olatunji, G.A., Kolawole O.M., Enonbun, K.I. Phytochemical screening and Antibacterial Activity of *Viscum album* (Mistletoe) Extracts. *Plant Sciences Res.*, 2008; **1**(3):44-46.
24. Onay-Ucar, E., Karagoz, A., Arda, N. Antioxidant activity of *Viscum album ssp. album. Fitoterapia*., 2006; **77**: 556-560.
25. Orhan D.D., Aslan, M., Sendogdu, N., Ergun, F., Yesilada, E. Evaluation of the hypoglycemic effect and antioxidant activity of three *Viscum album subspecies* (European mistletoe) in streptozotocin diabetic rats. *J. Ethnopharmacol*., 2005; **98**(1-2): 95-102.
26. Parekh, J., Jadeja, D., Chanda, S.V. Efficacuy of aqueous and methanol extracts of some medicinal plants for antibacterial activity, *Turk. J. Biol*., 2007; **29**: 203-210.
27. Prabuseenivasan, S., Jayakumar, M., Ignacimuthu, S. In vitro antibacterial activity of some plant essential oils, *BMC complementary and Alternative medicine*., 2006; **6**: 39.

28. Prashant, T., Kumar, B., Kaur, M., Kaur, G., Kaur, H.. Phytochemical screening and extraction. *International Pharmaceutica Science.*, 2011; **1**:1
29. Rizk, A.M. Constituents of Plants Growing in Qatar. *Fitoterapia*., 1982; **52**: 35-42.
30. Sadananda, T.S., Govindappa, M., Ramachandra, Y.L. Antibacterial activity of *Viscum album* endophytic fungal lectin. International Journal of Biological & Pharmaceutical Research., 2013; **4**(12): 1033-1042.
31. Siddiqui, A.A., Ali, M. Practical Pharmaceutical chemistry. Ist ed., CBS Publishers and Distributors, New Delhi. 1997; pp 126-131.
32. Sumathi, P., Parvathi, A. Antimicrobial activity of some traditional medicinal plants. *J. Med. Pl. Res*., 2010; **4**(4): 316-321.
33. Tyler, V. Phytomedicines in Western Europe: their potential impact on herbal medicine in the United States Herbalgram. 1994; **30**: 24-30.
34. Umoh, U.F., Ekpo, B.A.J., Bala, D.N., Udobang, J.A., Cocobassey, M., Etim, E.I. Phytochemical and comparative antidiabetic studies of leaf extracts of *Viscum album* from different plant hosts. *Int. J. Biol. Chem. Sci*., 2011; **5**(4): 1448-1454.
35. Westh, H., Zinn C.S., Rosdahl, V.T. An international multicenter study of antimicrobial consumption and resistance in *Staphylloccocus aureus* isolates from 15 hospitals in 14 countries. Microb. *Drug Resist*., 2004; **10**: 169–176.
36. Yusuf. L., Oladunmoye, M.K., Ogundare, A.O., Akinyosoye, F.A., Daudu, O.A.Y., Hassan, G.A. Antimicrobial and antioxidant properties of mistletoe (*Viscum album*) growing on cola (cola nitida) tree in Akure North, Nigeria. *Journal of Microbiology Research and Reviews*., 2003; **1**(3): 35-41.