

COMPARATIVE STUDY ON THE PHYTOCHEMICALS AND ANTIMICROBIAL POTENTIALS OF EXTRACTS OF *Moringa oleifera* and *Telfaria occidentalis* ON ANTIBIOTIC RESISTANT ORGANISMS

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Abstract

Resistance to antibiotics by etiologic microbial agents resulted in the quest for natural products with novel antimicrobial activity that will help to manage or combat the menace caused by resistant organisms. Therefore this study explores the phytochemicals of different extracts of Moringa oleifera and Telfaria occidentalis and their invitro biologic effect on antibiotic resistant organisms. The plants parts used for this study (M. oleifera leaves and seed and T. occidentalis leaves) were screened for phytochemical components from four solvents (ethanol, acetone, cold water and hot water). The results showed the presence of cardiac glycoside and polyphenols in all the extracts of M. oleifera seed and leaves respectively. Similarly, saponin was detected in all the extracts of T. occidentalis leaves and all the extracts of M. oleifera seed. Anthraquinone was not detected in the extracts of both plant parts. Other phytochemical components such as phlobatannins, alkaloids steroids and flavonoids were detected variably in both the plants parts extract. The antimicrobial activity of both plant parts were tested against multi-drug resistant Staphylococcus aureus, Salmonella typhi, Escherichia coli and Candida albicans at 100mg/ml concentration for all extracts. The tested organisms were resistant to cold and hot aqueous extracts of M. oleifera seed and also to cold aqueous extract of M. oleifera leaves. Also, all the tested organisms were not susceptible to hot aqueous extract of T. occidentalis leaves. However, the organisms were variably susceptible to ethanol and acetone extracts of M. oleifera with zones of inhibition which ranged from 12mm - 17mm for M. oleifera seed and 8 - 12mm for M. oleifera leaves. For T. occidentalis however, the cold aqueous extract exhibit higher (15 -19mm) antimicrobial activity against the tested organisms in comparison to ethanol (10 - 16 mm)and acetone (13 - 14 mm) extracts. The results further revealed that the antimicrobial activities of leaves and seed of M. oleifera on all the isolates were not statistically different (P=0.333) and were not significantly different from the extracts of T. occidentalis leaves (P=0.645). Based on solvents used, the antimicrobial activity of ethanol and acetone extracts of all the plants parts were not statistically different (P=0.799), but were significantly higher than that of cold aqueous extracts of all the plants parts (P=0.007). However, the antimicrobial activity of cold aqueous extracts of T. occidentalis leaves on all the isolates was not significantly different from that of acetone extracts (P=0.109) and ethanol extracts (P=0.065) of all the plants parts. This result therefore showed that the leaves and seed of M. oleifera and T. occidentalis leaves if harnessed and exploited adequately may be sources of antimicrobial agent and a potential remedy for the treatment of infections caused by the organisms under study.

Keywords: phytochemicals, antimicrobial activity, multi-drug resistant, Moringa oleifera, Telfaria occidentalis

Introduction

A medicinal plant is any plant in which one or more of its parts contains substances that can be used for the synthesis of useful drugs (World Health Organization, 1977). They are rich in biologically active chemical substances commonly known as phytochemicals (Sofowora, 1996). These phytochemicals exist secondary as metabolites in one or more of these plants (Kayode and Kayode, 2011) and have properties that are of interest in medicine. A large proportion of population in developing countries especially Nigeria depends largely

on traditional medicine derived from to folklore medicinal plants due perspectives, non-accessibility or cost of synthetic antimicrobials (Tula et al., 2014). Moringa oleifera a medicinal plant is a small or medium size, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark (Patel et al., 2014), found in many tropic and subtropics regions worldwide (Trapti, 2009). In Northern Nigeria, M. oleifera is highly valued because almost every part of the tree



is edible with high nutritional value. The plant is referred to number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and "Mothers best friend" (Julia, 2008). In Nigeria Moringa is called by different names based on ethnic groups; it is called Zogale (Hausa), Ewe igbale and Idagbo monoye (Yoruba), Ikwa oyibo (Igbo), Fuufu (Tula), Labele (Tangale). It is a drought tolerant plant that thrives best under the tropical climate and tolerates different soil types (Fahey, 2005). In addition, reports shown that the plant has been used extensively in traditional medicine for the treatment of several human diseases due to its antimicrobial properties, promotes digestion, as stimulant in paralytic afflictions, epilepsy and hysteria (Lockett et al., 2000; Anwar et al., 2007; Farooq et al., 2012; Mishra et al, 2011). The seed powder of *M. oleifera* works as a natural coagulant which clarifies very turbid water (Broin et al., 2002).

Telfairia occidentalis is a large perennial plant which climbs by means of bifid and tendrils which are usually coiled. The stem has five ridges often covered with multicellular hairs, especially when young. The leaves of the plant are compound; usually 3-5 foliate, with blades and petioles also covered with multicellular hairs. In different part of the world, T. occidentalis is called by different names as follows: Fluted pumpkin, oyster nut, oil nut, fluted gourd and Telfairia (English); Costillada nut (Spanish); Krobonko (Ghana); Oroko, pondokoko and Gonugbe (Sierra Leone). In Nigeria, it is called Ugwu (Igbo), Aworoko, Eweroko (Yoruba), umeke in Edo, Umee (Urhobo) and Ikong (Efik/Ibibio) (Akoroda, 1990). The plant occurs in the forest zone of West and Central Africa, most frequently in Nigeria Benin and Cameroon (Kayode and Kayode 2011). In Nigeria, it has been suggested that T. occidentalis originated from South east and distributed by the Igbos, who have cultivated this crop right from ancient times (Kayode and Kayode, 2011; Oyewale and Abalaka, 2012). The vegetable is very popular particularly in soup and folk medicine preparations. Reports have shown that the plant is useful in the treatment and management of various diseases such as anaemia, diabetes, chronic fatigue and gastrointestinal disorder (Oboh et al., 2006; Alada, 2000; Dina et al., 2006; Kayode and Kayode 2011). This study was therefore undertaken to explore the phytochemical constituents and antimicrobial activities of ethanol, acetone, cold and hot aqueous extracts of seed and leaves of M. oleifera

and leaves of *T. occidentalis* on antibiotic resistant clinical isolates.

Matreials and methods Collection of Plant materials

The leaves of *M. oleifera* and *T. occidentalis* were purchased from Mubi main market, while the seed of M. oleifera was collected in Vintim area of Mubi metropolis. Both plants parts were identified and authenticated the Botany in unit of Department of Biological Science Technology, Federal Polytechnic Mubi, Adamawa State.

Extraction Procedure

The *M. oleifera* (leaves and seed) and leaves of T. occidentalis were air-dried at room temperature and ground to fine powder. 50g of each of the powdered plant part was soaked in 200ml distilled water and was allowed to stand for 48h at room temperature after thorough vortexing. Each mixture was filtered using whatman no.1 filter paper. The filterate were concentrated in vacuo using rotary evaporator. Similar procedure was followed to obtain hot aqueous and ethanolic extracts of the plant parts using ethanol and hot water as extracting solvents. All the aqueous (hot and cold) and ethanolic dried extracts of the plant parts were stored in sample bottles at 4°C prior to use (Tula et al., 2014).

Phytochemical Screening

Qualitative phytochemical screening for the presence of phytochemical compounds were carried out on the aqueous (hot and cold), acetone and ethanol extracts of all the plants parts using standard as described by Sofowora (1993) and Evans (2002). The phytochemical components screened includes: Flavonoids. Alkaloids. Anthraquinones, Phlobatannins, Cardiac glycosides, Polyphenols, Saponins and Steroids.

Test Organisms

The test isolates used for this study were clinical isolates collected from New Life Hospital Mubi, Adamawa State. The antibiotic susceptibility testing of the clinical isolates was performed on nutrient agar plates by agar disk diffusion method as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002).These isolates include;

Staphylococcus aureus, Salmonella typhi, Escherichia coli and Candida albicans. Determination of Antibacterial Activity



The dried aqueous (cold and hot), acetone and ethanolic extracts of M. oleifera leaves and seed and T. occidentalis leaves were reconstituted in glycerol to obtain a final concentration of 100mg/ml for this test. The susceptibility test was done using agar well diffusion method. 0.1ml aliquot of each test suspension (equivalent organism to 0.5McFarland standards) was transferred onto dried agar plates in duplicate and was spread evenly with a sterile bent glass rod. After drying, three (3) wells were bored (using 6mm diameter cork borer) into the dried nutrient agar plates and 0.5ml each of the extracts (leaves stem bark and root bark) was aseptically introduced into two wells. Glycerol was introduced into the third well as control. The plates were then incubated at 37°C for 24h after which zones of inhibition were measured in centimetres and recorded appropriately (Tula et al., 2014)

Statistical Analysis

Non parametric Mann-Whitney statistics was used to compare the difference in antimicrobial activity between M. oleifera and T. occidentalis extracts. Whereas chi square was used to compare antimicrobial activity between the extracts of the different solvents used and also between extracts of the plants parts used. All statistical analyses were carried out using the SPSS 17.0 based program. Significance window difference and non- significance difference was defined when p≤0.05 and p>0.05 respectively.

Results

The antibiotic resistance profile of organisms used in this study as shown in Table 1 revealed that the organisms are resistant to 4-7 antibiotics with at least 2 class of antibiotics in each case.

The results of the phytochemical analyses showed that anthraquinones and phlobatannins are absent in leave and seed extracts of M. oleifera. Cardiac glycosides and saponins are present in all the seed extracts of M. oleifera but variable in the leaves extracts. Similarly, polyphenol is present in all leave extracts of M. oleifera. Although anthraquinones is absent in all the leave extracts of T. occidentalis, saponins is present in all the leave extracts of T. occidentalis. Polyphenol, alkaloid, steroid and flavonoid are variably present in both plants parts extracts (Table 2).

The antimicrobial activities of all the plants parts involved in this study were taken at

100mg/ml concentration for all extracts. The results as shown in Table 3 revealed that both cold aqueous extracts of seeds and leaves of *M. oleifera*, hot aqueous extracts of M. oleifera seed and T. occidentalis leaves had no inhibitory action on all the tested organisms. Consequently had no statistical difference from each other (P=0.154). The ethanol and acetone extracts of leaves and seed of *M. oleifera* produced impressive antimicrobial activity against the tested organisms with zones of inhibition ranged from 8-17mm in diameter. In the same vein, the acetone, ethanol and cold aqueous extracts of leaves of T. occidentalis had variable but impressive inhibitory effect on all the tested isolates with zones of inhibition ranged from 10-19mm in diameter. Salmonella Typhi was not inhibited by ethanolic extracts of seed and leaves of M. oleifera, while S. aureus was not inhibited by both ethanolic and acetone extracts T. occidentalis leaves. Similarly, acetone extracts of leaves of M. oleifera has no inhibitory effect on S. aureus. E. coli was resistant to all the leaf extracts of M. oleifera and to all the aqueous (hot and cold) extracts of both plants parts. Similarly, C. albicans was resistant to all the aqueous (hot and cold) extracts of M. oleifera leaf and seed and to the hot aqueous extract of T. occidentalis.

Based on plant types, the antimicrobial activities of extracts of *M. oleifera* and *T. occidentalis* on all the tested organisms were not significantly different (P=0.242).

Based on solvents used, the antimicrobial activity of ethanol and acetone extracts of all the plants parts were not statistically different (P=0.799), but were significantly higher than those of hot aqueous extracts of all the plants parts (P=0.007). However, the inhibitory effect of cold aqueous extracts of *T. occidentalis* leaves on all the isolates was not significantly different from that of acetone extracts (P=0.109) and ethanol extracts (P=0.065) of all the plants parts (Table 4).

Based on the plant parts used, the antimicrobial activities of extracts of M. *oleifera* seed on all the isolates were not statistically different from the extracts of M. *oleifera* leaves (P=0.333) and also from the extracts of T. *occidentalis* leaves (P=0.645) (Table 5).



Organisms	Resistant profile
Staphylococcus aureus	tet, amx, pen, chl.
Salmonella typhi	pen, chl, cfr, ofl, amx.
Escherichia coli	amx, pen, cef, cep, cfr, tet, ofl.
Candida albicans	cef, pen, amx, cfr.

Key: amx= amoxicillin, pen= penicillin, cef= cefozolin, cep= cephalexin, cfr= cefaraxine, tet= tetracycline, ofl= ofloxacin, chl= chloramphenicol

Table 2: phytochemical comp	oonents of Moringa oleifer	ra and Telfaria occidentalis
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Phytochemicals	Moringa oleifera						Telfaria occidentalis			talis		
		S	eed			Le	aves			Le	aves	
	EE	AE	CaE	HaE	EE	AE	CaE	HaE	EE	AE	CaE	HaE
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+	+	-	-	+	+	-	+
Phlobatannins	-	-	-	-	+	+	-	-	+	-	+	-
Polyphenols	+	+	-	+	+	+	+	+	-	+	+	+
Saponins	+	+	+	+	-	-	+	+	+	+	+	+
Alkaloids	+	-	+	+	-	-	-	+	+	-	+	+
Steroids	+	-	-	+	+	+	-	+	-	-	+	+
Flavonoids	+	-	-	-	-	-	-	+	-	-	-	+

Key: EE= Ethanol extract, AE= Acetone extract, CaE= Cold aqueous extract, HaE= Hot aqueous extract.

Table 3: Antimicrobial	l activity of	Moringa	oleifera	and Telfairia	occidentalis
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Test organisms	Zone of inhibition (mm)											
	Moringa oleifera					Telfairia occidentalis			talis			
		S	eed			Lea	aves			Lea	aves	
	EE	AE	CW	HE	EE	AE	CW	HE	EE	AE	CW	HE
Staphylococcus aureus	16	15	-	-	12	-	-	10	-	-	15	-
Salmonella Typhi	-	13	-	-	-	13	-	10	10	14	15	-
Escherichia coli	17	12	-	-	-	-	-	-	16	13	-	-
Candida albicans	14	12	-	-	8	8	-	-	15	-	19	-

Table 4: Comparison of the *P-values of the antimicrobial activity of extracts of Moringa oleifera and Telfairia
occidentalis based on solvents used

Solvent used	EE	AE	CaE	HaE
EE	1.000 ^a			
AE	0.799 ^b	1.000^{a}		
CaE	0.065^{b}	0.109 ^b	1.000 ^a	
HaE	0.007 ^c	0.014 ^c	0.357 ^b	1.000 ^a

(*P-value generated from chi-square Least Significant Difference, LSD)

KEY: EE=ethanol extracts, AE= acetone extracts, CaE= cold aqueous extracts, HaE= hot aqueous extracts, a=exactly the same, b=not significant (P>0.05), c=statistically significant (P ≤ 0.05).

Table 5: comparison of the *P-values of the antimicrobial activity of extracts of leaves and seed of *M. oleifera* and extracts of *T. occidentalis* leaves

Plant parts	MOS	MOL	TOL
MOS	1.000 ^a		
MOL	0.333 ^b	1.000 ^a	
TOL	0.645 ^b	0.165 ^b	1.000 ^a

(*P-value generated from chi-square Least Significant Difference, LSD)

KEY: MOS=*M. oleifera* seed, MOL= *M. oleifera* leaf, TOL= *T. occidentalis* leaf, \mathbf{a} =exactly the same, \mathbf{b} =not statistically significant (P>0.05).

Discussion

Medicinal plants produce a large diversity of secondary metabolites which are either used as precursors or lead compounds in the pharmaceutical industry (Shokeen et al., 2009). The preliminary phytochemical investigation showed that the leaf and seed of M. olefeira and leaf of T. occidentalis contains all the phytochemicals tested except anthraquinone that is absent in all the plants parts extracts and phlobatannin that is absent in the seed extracts of M. oleifera. This suggests that the detection of the phytochemicals in all the plants parts tested is solvent dependent. This opinion aligned with previous studies who opined that the extracts of plant parts are known to have biological properties and these are usually found to vary with the type of solvent used to extract the active components (Kalpana *et al.*, 2013; Patel *et al.*, 2014). In agreement with our findings, saponins was previously reported in ethanol extract of *M. oleifera* leaf (Bukar *et al.*, 2010), but contrary to our findings, the same study reported the presence of flavonoids in ethanol extract of *M. oleifera* leaf which is absent in our study. In the same vein our study reported the presence of alkaloid, saponins and flavonoid in ethanolic extract of *M. oleifera* seed which is in agreement with previous study (Bukar *et al.*, 2010).

In agreement with our findings, previous study reported the presence of alkaloids and saponins in both aqueous and ethanol extracts of *T. occidentalis* leaf (Oboh *et al.*, 2006). Contrary to our findings, previous



study reported of the presence anthraquinone Т. in leaf extract of occidentalis (Inuwa et al., 2012; Esevin et al., 2014). The same study reported the presence of alkaloids, saponins and steroids in the leaf extracts of T. occidentalis which is in agreement with the findings of this study.In addition, the absence of phenolic compounds (polyphenols and flavonoid) in ethanol extract of T. occidentalis leaves and the presence of the same compound in aqueous extract of the same plant part concurred with the report of previous studies which revealed that phenols present in T. occidentalis leaves are more soluble in aqueous solution than ethanol, consequently, the aqueous extract could be a more potent antioxidant than the ethanolic extracts (Oboh, 2006). Another study suggested that the level of phenol content in aqueous extract of T. occidentalis leaves could be responsible ethno-medicinal for the effectiveness of the plant part in the and or prevention management of haemolytic anaemia and diabetes (Oboh, 2004).

The plants under study are often used as vegetables. Vegetables constitute rich source of phytochemicals. These phytochemicals have a protective and therapeutic effect essential to preventing diseases and maintaining a state of wellbeing, by stimulating enzymes in the liver that render some carcinogens harmless and help the body stimulate others (Oboh *et al.*, 2006). Studies have shown that the risk of developing chronic diseases, such as cancer and cardiovascular diseases can be reduced by regular intake of vegetables and fruits (Liu, 2005; Oboh, 2006).

The variation of the phytochemicals as reported might be due to differences in geographical location in which the plant was grown physiological age of the plant, extracting solvents, composition of the

supporting soil, time of harvest of the plant material and method of preparation and extraction (Tula et al., 2014). In conformity with these, report from previous study variation in showed that great the composition and concentration of phytochemicals in different parts of plants is inevitable in varying geographical location or habitat (Farooq et al., 2007). More so, plants have different biological and chemically active constituents stored in them but their concentration in different parts of the plant may not be the same. Consequently, these active ingredients are released into solution in varving combination and strength (Thilza et al., 2010; Shokeen et al., 2009). Another finding revealed that phytochemicals play a vital role in plant defence (Shinwari et al., 2015). From these, we may conclude that phytochemicals are like antibodies in plants which are produced in response to foreign challenges; their types and concentrations all depends on the type of challenges encountered by the plant.

The results of the antimicrobial activity showed that acetone and ethanol extract of seed and leaf of *M. oleifera* showed varying degree of inhibitory effect on the tested organisms. In line with the findings of our study, several studies have also shown variable antimicrobial activity of M. oleifera parts on organisms that either act as potential human or food borne pathogens (Kalpana et al., 2013; Busani et al., 2012). The test organisms in the study are either Gram positive (S. aureus) or Gram negative (E.coli and S. Typhi). Consequently, the ability of the extracts of both plant parts to exhibit greater antibacterial activity against both gram positive and Gram negative bacteria is noteworthy. A previous study revealed that extracts from M. oleifera leaf showed greater antibacterial activity against Gram negative bacteria than gram positive



(Busani et al., 2012). On the other hand, many other studies opined that most plant extracts have more activity against Gram positive bacteria than Gram negative bacteria (Aiyegoro et al., 2008; Boussaada et al., 2008; Ashafa and Afolayan, 2009). Contrary to these opinions, our study portrayed similar trend of antibacterial activity between acetone and ethanol extracts of M. oleifera against both Gram positive and Gram negative bacteria. The ability of acetone extracts of M. oleifera seed to inhibit S. Typhi and E.coli, ethanol extract of M. oleifera seed to inhibit E. coli and acetone extract of M. oleifera leaf to inhibit S. Typhi with variable zone of inhibition is highly remarkable. This is because these organisms are gram negative bacteria which have records of multiple antibiotic resistant traits (Boussaada et al., 2008) as confirmed in our study by their resistant profiles. Several studies have shown that these bacteria are generally less sensitive to the activity of plant extracts (Pintore et al., 2002; Wikinson et al., 2003, Boussaada et al., 2008). In this study however, S. Typhi showed no susceptibility to ethanolic extract of M. oleifera seed. This is in conformity with previous findings (Jamil et al., 2007; Lar et al., 2011; Mishra et al., 2011; Farooq et al., 2012).

The non activity of hot and cold aqueous extracts of *M. oleifera* seed and leaf and hot aqueous extract of *T. occidentalis* leaf against all the organisms tested in this study is in agreement with previous findings which revealed that aqueous extracts of plants generally exhibits little or no antimicrobial activities (Aiyegoro *et al.*, 2008; Ashafa *et al.*, 2008; Rahman *et al.*, 2009; Lar *et al.*, 2011; Busani *et al.*, 2012). In this study, both Gram positive and Gram negative organisms are resistant to aqueous extracts at the same rate. This is contrary to previous findings which showed that Gram

negative bacteria are less susceptible to aqueous extracts of plants (Kuhnt *et al.*, 1994; Afolayan and Meyer, 1995). The nonactivity of aqueous extracts on the tested organisms as shown in this study might be due to the fact that aqueous extracts do have many different compounds that may interact antagonistically in their activities. More so, active principles from plant materials are readily extractible in organic solvents than aqueous solvents (Eloff, 1998). Contrary to the findings of this study, a previous study showed that *M. oleifera* aqueous extracts had antimicrobial activity (Dahot, 1998).

The antifungal activities of *M. oleifera* leaf and seed have been variably reported (Nwosu and Okafor, 1995; Raheela *et al.*, 2008). In this study, ethanol and acetone extracts of *M. oleifera* leaf and seed exhibit antifungal effect against *C. albicans*, whereas all the aqueous extracts did not. Previous studies have shown that aqueous and ethanol extract (Patel *et al.*, 2014) and aqueous and acetone extracts (Busani *et al.*, 2012) of *M. oleifera* leaves did not exhibit antifungal activity against *C. albicans*.

The finding of this study also portrayed the efficacy of ethanol extract of T. occidentalis leaf on S. typhi and E. coli. This is similar to previous report (Oyewale and Abalaka, 2012) on the effect of T. occidentalis leaf extract against selected intestinal pathogens. Previous study has shown that methanol extract of T. occidentalis leaf had inhibitory effect on S. aureus and E. coli (Nwankanma et al., 2014). Contrary to this report, all the organic solvents (ethanol and acetone) extracts of T. occidentalis leaf used in this study had no inhibitory effect on S. aureus, but similar to our findings had inhibitory effect on E. coli and S. Typhi. In our study however, cold aqueous extracts of T. occidentalis remarkable exhibit antimicrobial activity against Gram positive, Gram negative and C. albicans in a manner



that is not significantly different from those of acetone and ethanol extracts of both plants parts. This might be due to the fact that the active principles in the leaves of *T*. *occidentalis* are more soluble and extractable in aqueous solution than organic solvents as previously reported (Oboh, 2006).

The variation in antimicrobial activity could be attributed to differences in location, season, and physiological stage of the plants (Taylor and Vanstaden, 2001). These factors to affect the are known chemical composition and amount of active compounds in plants which is believe to contribute immensely to the biological activity of the plants.

Conclusion

Traditionally, plants have provided a source of hope for refreshingly new and interesting drug compounds, because plant herbal mixtures have made large contributions to

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human health and well-being. In recent times, plants are still indispensable as they constitute the major key components of today's pharmaceuticals. Moringa oleifera and T. occidentalis plants parts are rich in a wide variety of phytochemicals. These phytochemicals are the secondary metabolites usually found in plants and perform specific functional roles especially of therapeutic importance. With the incidence of multidrug-resistant pathogens and the menace they constitute to therapy, plants and their derivatives are being investigated as possible solutions. Therefore, the results of this study showed that the leaves and seed of M. oleifera and leaves of T. occidentalis if harnessed and exploited adequately may be sources of antimicrobial agent and a potential remedy for the treatment of infections caused by the organisms under study.

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