

Original Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF THUJA OCCIDENTALIS SEEDS EXTRACTS AGAINST THE ISOLATED COMMON SKIN INFECTING MICROORGANISMS

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ABSTRACT

Objective: This study represents the first attempt to investigate the antimicrobial activity of *Thuja occidentalis* seeds extracts against the isolated skin infecting microorganisms.

Methods: *T. occidentalis* seeds were powdered and extracted continuously by Soxhlet apparatus using 96% ethanol to obtain the total crude ethanolic extract. The extracts with increasing polarity were successively prepared with petroleum ether, chloroform, ethyl acetate, and methanol using the Soxhlet apparatus.

The extracts were screened for their phytochemical constituents. Their antimicrobial activity against standards microorganism and the isolated skin infecting microorganisms was evaluated using the agar diffusion method.

Results: The result revealed the presence of flavonoids, alkaloids, terpenoids, tannins and cardiac glycosides. The antimicrobial activity result showed that *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* were found to be sensitive to the extracts of *T. occidentalis* seeds with inhibition zones range between 20±0.57-12±1.25 mm. The isolated skin infecting microorganisms; *Staphylococcus aureus* and *Staphylococcus epidermidis* were found to be highly susceptible to the extracts of *T. occidentalis* seeds with inhibition zones of 28±1.02-22±0.45 mm and minimum inhibitory concentration (MIC) values of 6.25 mg/ml.

Conclusion: From the results it concludes, *T. occidentalis* seeds extracts had a considerable level of antimicrobial activity. They are more effective towards gram-positive than gram-negative bacteria. Hence, the active principles present in the extracts are particularly powerful effective against the skin infected isolated bacteria. In conclusion, there is a proportional increasing in the antimicrobial activity with increase of the solvents polarity. This may attribute to the fact that the chemical constituents responsible for the antimicrobial activity are of high polarity, such as tannins.

Keywords: *Thuja occidentalis*, Phytochemical Screening, Antimicrobial Activity, Isolated, Common Skin, Infecting, Microorganisms

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INTRODUCTION

The plants are of great importance to the health of individuals and communities whereas, about 80% of world's populations rely on traditional medicine for their primary healthcare needs and the most of this therapy use plant extracts and their active compounds. The various type of secondary metabolites produced by medicinal plants such as alkaloids, flavonoids, steroids, phenolics, terpenes, volatile oils and other are well known for their medicinal use in traditional systems of medicine, folk medicines, modern medicines and pharmaceutical intermediates. Nowadays plant-derived substances have been of great interest owing to their versatile applications in the primary health care needs [1, 2].

Thuja occidentalis is one of the great importance in traditional medicine which it is widely used as a medicinal plant since 16th century [3]. *T. occidentalis* belongs to Cupressaceae family, and commonly known as *Thuja* is an evergreen coniferous tree use traditionally for treating of many diseases, mainly use as antimicrobial agent to treat skin infection; it has been used to treat conditions such as bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism [4]. It is well known as antimicrobial, anticancer, analgesic and insecticide agent [5-8].

Skin infections such as abscesses, carbuncles and furuncles are known to be caused by several organisms whereas, *Staphylococcus aureus* is considered to be an almost-universal cause of several skin conditions. It is considered to be the most common organism implicated in both skin and soft tissue infections [9, 10]. In addition to *Staphylococcus epidermidis* is commonly associated with skin infections [11].

According to the traditional use of *T. occidentalis* in the treatment of skin infections, in addition to taking into account the universal and common agents cause skin infection the present study was aimed, at

the extraction of phytochemicals from *T. occidentalis* and evaluation of their antibacterial against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Firstly the screened of its general antimicrobial activity against the common standard microorganisms (gram -ve bacteria, gram +ve bacteria and fungus) must be done.

MATERIALS AND METHODS

Plant material collection and preparation

Dried seeds of *T. occidentalis* were collected from the local market at Omdurman, Sudan in October 2015 as it was recommended by several traditional plant users for treating several dermatological conditions. This was then authenticated by taxonomist at the National Centre for Research/Sudan. The voucher specimens; IKR2, October-2015 were deposited at Pharmacognosy Department, Faculty of Pharmacy, University of Medical Science and Technology (UMST). The collected specimen was preserved for further studies.

Preparation of *T. occidentalis* seeds extracts

The collected plant samples were cleaned, grounded into fine powder and used for the preparation of five extracts of *T. occidentalis*. Hundred gm of plant powder was extracted with 96% ethanol using Soxhlet apparatus at 40 °C for 48 h. to prepare the total crude ethanolic extract. Other 100 gm of powder was extracted successively with solvents of increasing polarity; petroleum ether, chloroform, ethyl acetate and methanol. All extracts were evaporated to dryness by a rotary evaporator, their percentage yields were determined and then stored at 4 °C for further investigation.

Phytochemical screening test

The phytochemical constituents of the extracts were detected using standard procedures as described by Trease and Evans [12] and Sofowora, [13].

Collection and preparation of the isolate bacteria

The skin infected samples were collected from the Khartoum Dermatological Diseases Hospital/Sudan, and the bacteria were isolated and identified by a microbiologist at the National Centre for Research/Sudan. The isolated bacteria were cultured in agar media and incubated for 24 h at 37 °C, and then the antimicrobial test was conducted.

Antimicrobial bioassay

Antimicrobial testing was carried out using agar diffusion method with some minor modification [14]. Nutrient agar media was used as a growth medium for the bacterial organisms. Another growth medium composed of sabouraud dextrose powder was prepared for promoting fungal growth. The media was placed in an autoclave using the following conditions 37 °C and 15 lbs pressure for 15 min for sterilization. The media (20 ml) was then poured in a petri dish under aseptic conditions and (0.2 ml) of the intended microorganism was introduced into prepared media. After the media had solidified, two discs containing the prepared extracts of *T. occidentalis* were added in each plate. Hence, 5 different discs were

prepared using the 96% ethanol total extract, petroleum ether, chloroform, ethyl acetate and methanol extracts. The experiment was performed in triplicate,

Determination of the minimum inhibitory concentrations (MIC)

The antimicrobial activity of *T. occidentalis* extracts with serial dilution of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25% mg/ml were evaluated using agar diffusion method [14] to determine the minimum inhibitory concentration (MIC) of the extracts sufficient to prevent the growth of the skin infected microorganisms.

RESULTS

Percentage Yields

T. occidentalis seeds extracts produced by using of various solvents were found to be of quite variable percentages yields (table 1). Less polar solvents were possessing modest values, such as petroleum ether (1.3%) and chloroform (4.1%), whereas, the more polar solvents; ethyl acetate (64.9%) and methanol (15.2%) had more substantial values. The total ethanol (96%) extract had a percentage yield of 73.7%.

Table 1: Percentage yields of *T. occidentalis* seeds extracts

Plant extract	Extract yield (g/100g)	Percentage yield (w/w)
Total (crude) ethanol	73.7	73.7%
Petroleum Ether	1.3	1.3 %
Chloroform	4.1	4.1%
Ethyl acetate	64.9	64.9%
Methanol	15.2	15.2%

Phytochemical screening

The phytochemical screening of *T. occidentalis* seeds extracts (table 2) revealed the presence of tannin in all assessed extracts.

Alkaloid was absent in petroleum ether and ethyl acetate extracts. Carbohydrates, steroids and saponins were not detected in any of the extracts.

Table 1: Qualitative phytochemical screening of *T. occidentalis* seeds extracts

phytochemicals	Total ethanol extract	Petroleum ether extract	Chloroform extract	Ethyl acetate Ether	methanol extract
Carbohydrates	-	-	-	-	-
Reducing Sugar	+	-	-	+	+
Compound Reducing sugar	+	+	-	+	+
Flavanoids	+	-	+	+	+
Tannins	++	±	+	+	++
Alkaloids	+	-	+	-	+
Sterols	-	-	-	-	-
Cardiac Glycosides	+	-	-	+	+
Terpenoids	+	-	-	+	+
Saponins	-	-	-	-	-

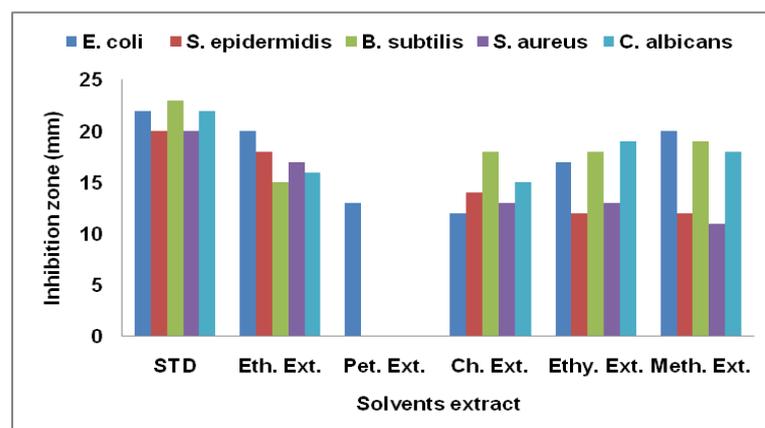


Fig. 1: Antimicrobial activity of *T. occidentalis* seeds extracts against standard tested microorganisms, STD= standard streptomycin, Eth Ext= total (crude) ethanol extract, Pet Ext= petroleum ether extract (successive), Ch Ext= chloroform extract (successive), Ethy Ext= ethyl acetate extract (successive), Meth Ext= methanol extract (successive). The values represent the mean of the three determinants

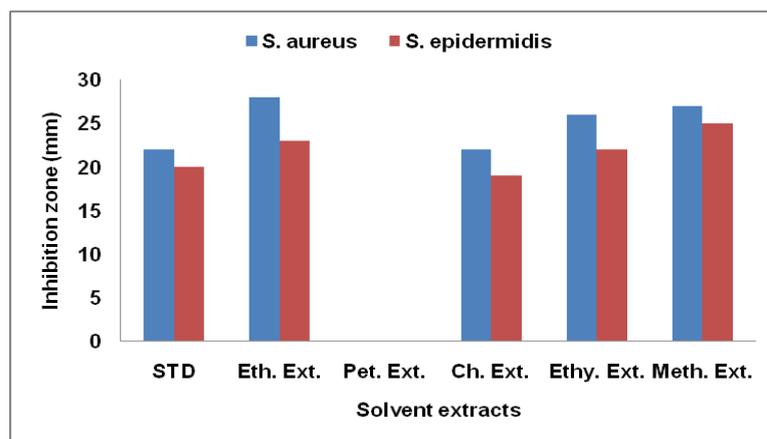


Fig. 2: Antimicrobial activity of *T. occidentalis* seeds extracts against isolated bacteria, STD= standard Streptomycin, Eth Ext= total (crude) ethanol extract, Pet Ext= petroleum ether extract (successive), Ch Ext= chloroform extract (successive), Ethy Ext= ethyl acetate extract (successive), Meth Ext= methanol extract (successive). The values represent the mean of the three determinants

Antimicrobial activity

The antimicrobial activity of *T. occidentalis* seeds extracts, against standard microorganisms (fig. 1) was found to be highest against *E. coli* followed by *B. subtilis*, *C. albicans*, *S. aureus* and *S. epidermidis*. The highest activity was shown by an alcoholic (ethanol, methanol) followed by the ethyl acetate, whereas, the moderate activity showed by the chloroform extract. The petroleum ether had the least activity, only *E. coli* was found to be sensitive with low inhibition zone.

Regarding the activity against the isolated *S. aureus* and *S. epidermidis* bacteria (fig. 2), the two bacteria were highly sensitive to the ethanol, methanol and ethyl acetate extracts, and their activity was found to be higher than that of standard Streptomycin. The chloroform extract showed moderate antibacterial activity against the isolated bacteria. The two bacteria were resistant or insensitive towards the petroleum ether extract.

DISCUSSION

The diverse health benefit of the plants is usually known to be through the numerous phytochemicals extractions [1]. The percentage yield of *T. occidentalis* seeds extracts was found to be different with solvents used in the extraction. The low polar solvents revealed percentage yield less than the solvents with high polarity. These results clearly indicate that *T. occidentalis* contains a great deal more of polar compounds than non-polar compounds. The total ethanol (96%) extract showed percentage yield of 73.7% 9 (w/w), being greater than any of the successive extracts. This may be attributed to the fact that, the 96% ethanol is a universal solvent possess polar and non-polar moieties, and hence when used solely as an extraction solvent was capable of extracting the widest variation of chemical compounds.

The preliminary phytochemical analysis of *T. occidentalis* seeds extracts (table 2) revealed the presence of secondary metabolites which are important for medicinal activity. The screening revealed the presence of tannin in all assessed extracts, it was found to be highly concentrated in alcoholic (ethanol and methanol) extracts. Whereas, in the petroleum ether extract tannin was found in slightly amount, addition to the absence of the all other tested phytochemicals. Alcoholic (ethanol and methanol) extracts revealed the presence of all tested phytochemicals except carbohydrates, sterols and saponins which were found to be absent in all assessed extracts. The ethyl acetate extract revealed the presence of phytochemicals the same as in alcoholic extracts with the slightly difference in the absence of alkaloids. Addition to tannin the chloroform extract showed the presence of flavonoid and alkaloids only. The presence of tannins, flavonoids, alkaloids and terpenoids which are well known for their antimicrobial activity [1, 15] suggest

that *T. occidentalis* seeds extracts are endowed with beneficial antimicrobial effect and could serve as a base of future drugs.

The present study represents a broad spectrum antimicrobial activity of *T. occidentalis* seeds extracts, although the degree of susceptibility differs between different organisms. Inhibition of microorganisms by *T. occidentalis* seeds extracts, against standard microorganisms (fig. 1) was in the order of alcohol, ethyl acetate, chloroform and petroleum ether extracts. Highest was against *E. coli* followed by *B. subtilis*. The MICs of *T. occidentalis* extracts against standard tested microorganisms; *P. aeruginosa*, *E. coli*, *B. subtilis*, *S. aureus* and *C. albicans* were found to be 6.25 mg/ml, 25 mg/ml, 25 mg/ml, 12.5 mg/ml respectively for the ethanol, methanol and ethyl acetate extracts. For the chloroform extract was 6.25 mg/ml for *P. aeruginosa* and 12.5 mg/ml for the other.

Regarding the antimicrobial activity results of *T. occidentalis* seeds extracts against the isolated skin infecting bacteria (fig. 2), both isolated bacteria; *S. aureus* and *S. epidermidis* were highly sensitive towards the extracts excluding the petroleum ether fraction. The activity order was similar to that observed against standard microorganisms with MIC value of 6.25 mg/ml against *S. aureus* and *S. epidermidis* for ethanol, methanol and ethyl acetate, whereas, chloroform showed MIC values of 25 mg/ml against the two isolated bacteria. The results of *T. occidentalis* extracts against the two isolated bacteria, which are well known for their causing of skin diseases and infections [1, 10, 16, 17] compatible with antibacterial activity of the plant [18] and confirm the traditional used of the plant for the treatment of skin diseases and infections [4].

Based on the polarity of solvents and the phytochemical screening (table 2), the observed activity displays a wide variation in correlation with the polarity of extract solvents used, and the activity was found to be proportional to the polarity of the solvents, as well as the tannin concentration which was well known for the antimicrobial activity [19-25]. This may be attributed to the fact that the chemical constituents responsible for the antimicrobial activity are of high polarity, the main of such compounds are tannins. Finally, it should be stated that preliminary phytochemical analysis results obtained seem to comply with the antimicrobial activity results.

CONCLUSION

Phytochemical study of different solvent extracts of *T. occidentalis* seeds revealed the presence of alkaloids, terpenoids, flavonoids and tannins. In the present study, it was clear that ethanol and methanol extracts of *T. occidentalis* seeds contains an abundant amount of tannins which were well known for their antimicrobial effect. Therefore, further research is needed to isolate, purify and characterize the bioactive compounds of the plant.

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AUTHORS CONTRIBUTION

Design of the work, interpretation of data and writing and correction of the manuscript was done by the first author Dr. Ikram Mohamed Eltayeb. The experimental part of the work was done by Mr. Abdeen Salim Hamid.

CONFLICT OF INTERESTS

Declared none

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