wjpls, 2017, Vol. 3, Issue 10, 97-103 **Research Article** **ISSN 2454-2229**

World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *THUJA OCCIDENTALIS* EXTRACT AGAINST SOME HUMAN PATHOGENIC BACTERIA

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Article Received on 04/10/2017 **Article Revised on 25/10/2017** **Article Accepted on 15/11/2017**

ABSTRACT

The present study was conducted to evaluate the antimicrobial activity of methanolic extract of *Thuja occidentalis*. In vitro antimicrobial activity was tested by agar well diffusion assay against human pathogenic microbes which are gram negative bacteria; *Acetobactor, Citrobactor and E. coli* and gram positive bacteria; *Bacillus subtilis, S. aureus and Enterobacter* and fungi (*Rhizopus orgae, Ponicillium griseofulvm, Fusarium solani* and *Alterneria alternate*) were evaluated. This study show that methanolic extract of *Thuja occidentalis* inhibits the growth of micro organism dose dependently. A significant correlation was observed between zone of inhibition and concentration of extract. These results confirm the antimicrobial activity of *Thuja occidentalis* and support the traditional use of the plant in therapy of bacterial infection. These promising findings suggest the presence of antibacterial activity in the tested plant material, exhibited by its bioactive compounds, and serving them as an alternative antimicrobial agent. Thus, Thuja *occidentalis* could be considered as potential source of natural antimicrobials used for the treatment of bacterial and fungal infections.

KEYWORDS: Antimicrobial activity; disk diffusion method; methanol extract; Thuja *occidentalis*; pathogenic bacteria, fungi.

1. INTRODUCTION

Plant material based traditional medicines are readily available in rural areas at relatively cheaper than modern medicine (Mann et al., 2008). This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi et al., 2007). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento et al., 2000). In general, these plants are used in folk medicine in the treatment of skin diseases, venereal diseases, respiratory problems and nervous disorders. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Edeoga et al., 2005). Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe et al., 1998). Recently, interest in a large number of traditional natural products has increased (Thongson et al., 2004). Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis, 1994). Also antibiotics and other synthetic drugs show adverse effects on host like ulcers, hypersensitivity etc. besides their high cost. So, a retrospection of the healing power of plants and a return to natural remedies is an absolute need of our time. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy et al., 2001; Ateb and Urul, 2003). Due to the above reasons, herbal extracts of Thuja orientalis was tested against selected human pathogenic strains that is B. subtilis (causal organism of dysentery), S. aureus (responsible for nausea, vomiting, diarrhea and dehydration), Pseudomonas aeruginosa (infects the pulmonary tract, urinary tract, burns, wounds and causes blood infections), Klebsiella pneumoniae (causal organism of pneumonia), Alcaligens faecalis (nosocomial septicaemia in immunocompromised patients) and two fungal strains viz. Aspergillus niger (allergic bronchopulmonary aspergillosis) and Aspergillus flavus (released aflatoxin B1 which is a potent liver carcinogen). Thuja occidentalis has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism (Chang et al., 2000). Thuja occidentalis and

Thuja orientalis are two different species belonging to the same family Cupresseaceae. Thuja occidentalis are slow-growing trees native to North America (also known as American cedar) and grows in dense wet forests in South Eastern Canada and the North Western United States. It grows up to a height of about 60 ft (18.2 m), with trunks between 12 and 24 in (31 and 61 cm) in diameter. On the other hand, Thuja orientalis was an endemic tree of North Western China (also known as Chinese cedar) but now naturalised as an introduced species in Asia: eastward to Korea and Japan; southward to Northern India; and westward to Northern Iran. It is a short tree growing to a height of only 12–20 ft (4–6 m), and is sometimes used to form hedges as it tolerates pruning. Chinese cedar, however, is not as hardy as its North American counterparts (http://www.altmd.com/Articles/Thuja--Encyclopedia-ofAlternative-Medicine). The essential oil from seed coats of T. orientalis was screened for antimicrobial activity by Jain and Garg et al. (1997) while its antioxidant activity was studied by Duhan et al. (2013). Based on the above fact, in the present study, various extracts of stem and leaf of *Thuja occidentalis* were screened for their antimicrobial activity. The results of this study may further strengthen the recommendation for the use of ethno medicine in the treatment and control of microbial infections.

2. MATERIALS AND METHOD

Extract preparation

Thuja occidentalis **(**100 g**)** was defatted with petroleum ether (1000 ml) and the residue was extracted in 50% methanol with the help of soxhlet extraction unit. The sample was collected and concentrated in water bath at 40-50^{o}C and dried in hot air oven at 40^{o}C. The dried powder was kept in air tied box.

Microorganism

The test organism included the gram negative bacteria; *Acetobactor, Citrobactor and E. coli* and gram positive bacteria; *Bacillus subtilis, S. aureus and Enterobacter* fungi (*Rhizopus orgae, Ponicillium griseofulvm, Fusarium solani* and *Alterneria alternate*). All the bacterial and fungi strain was obtained from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37^{o}C and maintained on nutrient agar slant at 4^{o}C.

Antibacterial Assay

Agar well diffusion assay described by Perez *et al*. (1990) was used for testing antibacterial and antifungal activity. Antibacterial test was done by preparing bacterial suspension followed by the disc diffusion test. Antibacterial activity of Nigella sativa extract was determined by agar disk diffusion method at four different concentrations i.e., 100, 75, 50 and 25 mg/ml. Muller Hinton agar was prepared according to the manufactur's instruction and the plates were seeded with appropriate micro organism *(*Gram negative bacteria; *Acetobactor, Citrobactor and E. coli* and gram positive bacteria; *Bacillus subtilis, S. aureus and Enterobacter) and* fungi (*Rhizopus orgae, Ponicillium griseofulvm, Fusarium solani* and *Alterneria alternate*). Discs of 6 mm diameter were prepared from Whatmann filter paper No. 1 and sterilized. The discs were than impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (TE- Tetracycline, OF- Ofloxacin, AZ-Azithromucin and PC- Pipracillin) and Gram negative (Fu- Nitrofurantoin, GM- Gentamicine, CX- Cefotaxime and NF- Norfloxaci,5 µl/disc) bacteria were used as standard. The plates were incubated at 37^{o}C for 24 hrs and the zone of inhibition was measured with measuring scale. This experiment was carried out in triplicate for their confirmation.

3. RESULTS

3.1 Antimicrobial Activity

The initiation of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study shows that antibacterial activity of 50 % methanolic extract of *Thuja occidentalis* against *B. subtilis* is best in 100 % concentration after 12 hrs. (18.23±0.23mm zone of inhibition). Although 75% concentration is having mild effect as 14.02±0.15 mm zone of inhibition. In *Citrobacter* 100% concentration of extract is having good antibacterial activity at maximum zone of inhibition 19.23±0.87 mm. On the other hand 75 % is showing static activity from, with zone of inhibition of 16.22±0.23 mm. For *E. coli* 100% concentration of extract show zone of inhibition 13.58±0.25 mm. Although the same effect of 75% concentration of extract is also revealing as showing zone of inhibition 12.74± 0.65 mm. In the case of *Acetobacter* 75% and 100% concentration of extract show good activity with zone of inhibition of 14.52±0.45 mm and 13.20±0.45 mm respectively.

The above observations suggest that different concentration (50 %, 75 % & 100 %) were having good antibacterial activity against *Acetobacter, Citrobacter, E. coli, B. subtilis, S. aureus and Enterococcus.* Thus the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Norfloxacin and Ofloxacin. But extract is not potent than erythromycin and amoxicillin in these conditions (Table 1 and 2).

Table 1: The study of anti-bacterial activities of standard antibiotics using disk diffusion method.

Sl	Name of Bacteria	Zone of Inhibition (In MM)			
1	**Gram Negative (-)**	**TE10**	**OF5**	**AZ15**	**PC5**
	Acetobacter	18.22±0.98	16.66±1.10	12.12±0.25	12.56±0.23
	Citrobacter	17.12±0.76	18.45±1.23	15.56±0.76	17.45±1.10
	E. coli	14.52±0.23	16.56±0.54	19.23±1.02	13.56±0.23
2.	***Gram Positive (+)***	**FU10**	**GM5**	**CX15**	**NF5**
	Bacillus subtilis	11.15±0.25	12.54±0.68	09.56±0.23	14.25±1.65
	S. aureus	16.45±1.23	14.92±0.12	19.45±0.56	17.69±0.56
	Enterobacter	16.66±1.04	16.89±1.01	14.10±0.23	18.55±0.23

Table 2: The study of anti-bacterial activities of *Thuja occidentalis* extracts using Disk Diffusion method third observation table (Mean ± SE)

Sl	Bacterial Stain	Bacteria Use	Zone of Inhibition (In MM)			
			25%	50%	75%	100%
1	Gram Negative (-)	*Acetobacter*	09.33±0.87	14.23±1.02	14.52±0.45	13.20±0.45
		Citrobacter	11.10±0.56	08.58±0.96	16.22±0.23	19.23±0.87
		E. coli	07.98±0.23	11.33±1.10	12.74± 0.65	13.58±0.25
2	Gram Positive (+)	*B. subtilis*	11.23±0.98	13.58±0.58	14.02±0.15	18.23±0.23
		S. aureus	08.23±0.78	05.98±0.78	15.63±0.45	14.85±0.12
		Enterobacter	06.25± 0.54	09.74±0.45	06.33±0.36	10.20±0.78

3.2 Antifungal Activity of *T. occidentalis* Extract

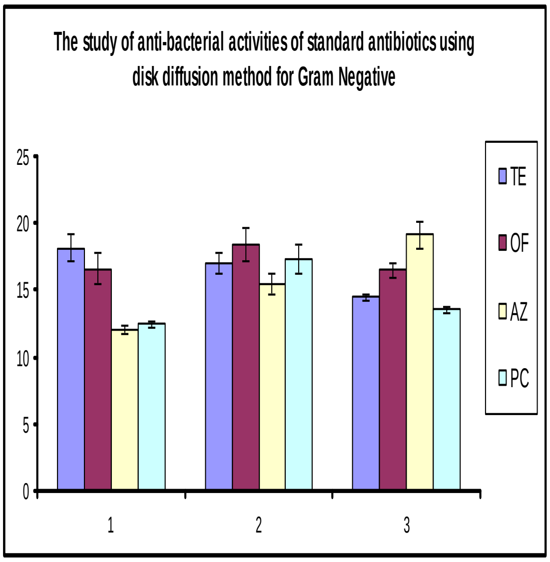
The antifungal activity of *T. occidentalis* against different types of fungi. The best zone of inhibition against *Rhizopus orgae* in 100% and 50% concentration of extract is 12.3±0.032 and 12.3±0.033, in 75% concentration of extract that is 11.6±0.033 and 25% concentration is 9.6±0.06. For best zone of inhibition aganist *Ponicillium griseofulvm* in 100% concentration of extract is 7.0±0.057, in 75% and 25% concentration of extract is 06±0.057 & 06±0.087 mm and 50% concentration of extract is 5.0 ±0.057. *Alterneria alternate* 100% and 75% concentration of extract is having good antifungal activity at maximum inhibition of 10.6±0.0008 and 8.0±0.01. Although 50% zone of inhibition is 7.0 ±0.057 & 25% is having 6.0 ±0.057 concentration of extract. In *Fusiarium solani* 100% concentration shows good activity of zone of inhibition 05.6±0.033, 75% concentration is 4.6±0.033 and 50% 4.3±0.017 zone of inhibition and 25% constrictions of extract having 0 zone of inhibition (Table 3 and 4).

Table 3: Showing the study of antifungal activity of standard antibiotics using Disk Diffusion Method.

No.	Name of Micro-organism (Fungi)	Zone of inhibition (in mm)			
		NX10	OF5	E15	CFM5
1.	*Rhizopus orgae*	12.00±0.23	12.00±0.87	11.00±0.74	09.00±0.87
2.	*Ponicillium griseofulvm*	11.00±0.12	10.00±0.89	09.00±0.23	11.000±1.03
3.	*Fusarium solani*	15.00±0.45	13.00±0.78	14.00±0.56	12.00±0.45
4.	*Alterneria alternate*	10.00±0.23	09.00±0.45	07.00±0.46	08.00±0.78

Table 4 Showing the study of antifungal activity of *Thuja occidentalis* extract using Disk Diffusion Method (Mean±SE).

No.	Name of Micro-organism (Fungi)	Zone of inhibition (in mm)			
		100%	75%	50%	25%
1.	*Rhizopus orgae*	12.3±0.032	11.6±0.032	12.3±0.066	09.6±0.033
2.	*Ponicillium griseofulvm*	07.0±0.057	06.0±0.087	05.0±0.057	06.0±0.057
3.	*Fusarium solani*	05.6±0.033	04.6±0.033	04.3±0.017	0
4.	*Alterneria alternate*	10.6±0.003	08.0±0.10	07.0±0.057	06.0±0.057



The study of anti-bacterial activities of standard antibiotics using disk diffusion method for Gram Negative
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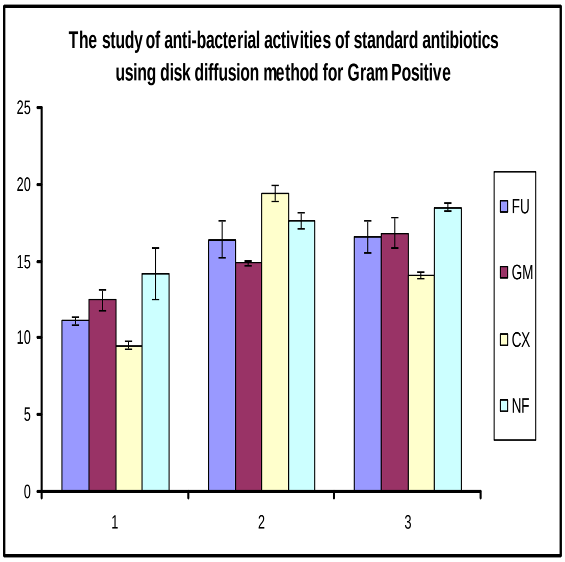

The study of anti-bacterial activities of standard antibiotics using disk diffusion method for Gram Positive
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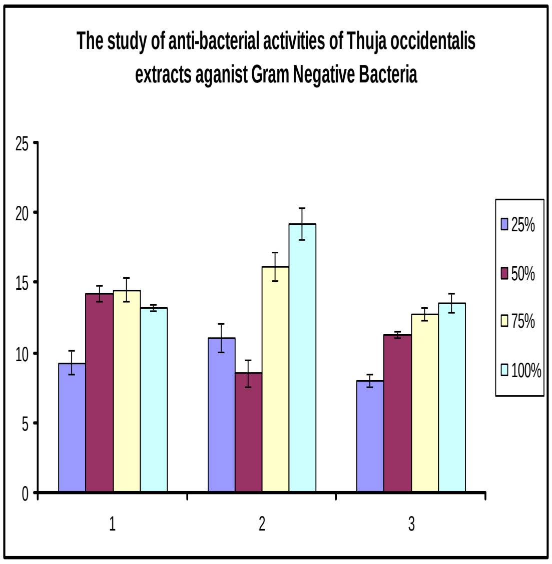

The study of anti-bacterial activities of Thuja occidentalis extracts aganist Gram Negative Bacteria
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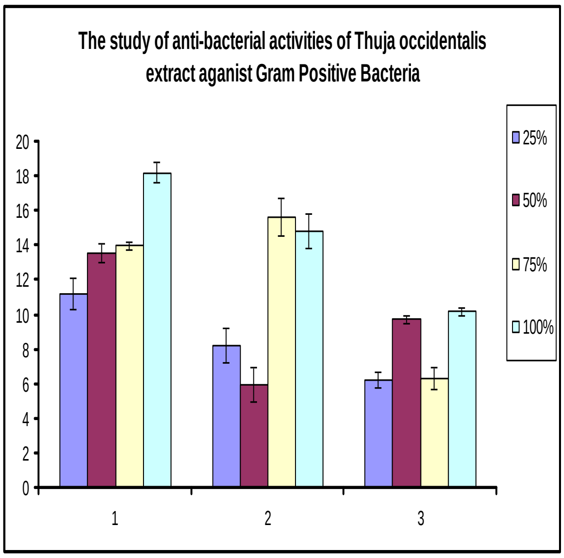

The study of anti-bacterial activities of Thuja occidentalis extract aganist Gram Positive Bacteria
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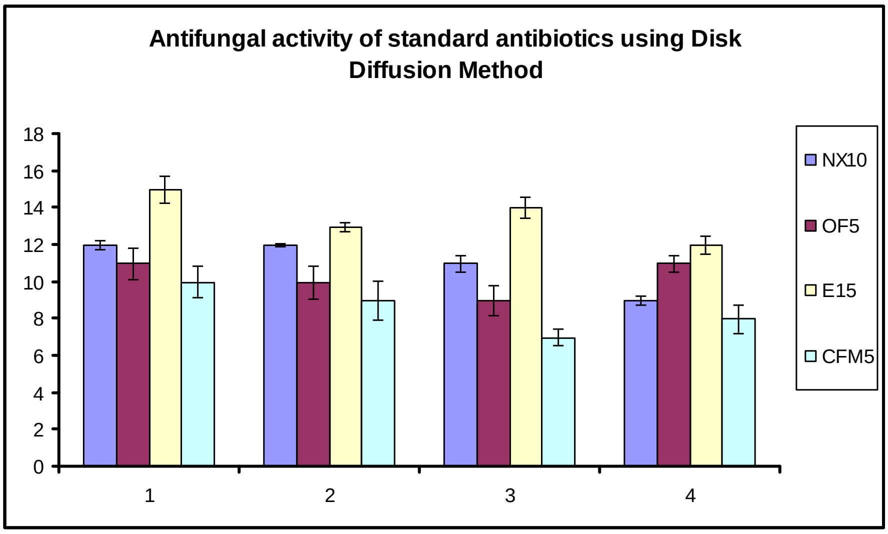

Antifungal activity of standard antibiotics using Disk Diffusion Method
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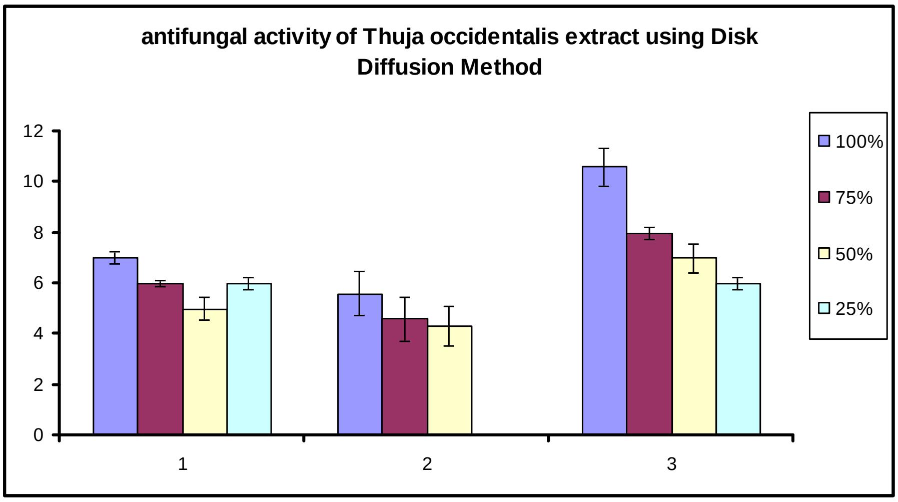

4. DISCUSSION

In traditional medicine system of India has been widely used as remedy for a variety of disorders such as emetic, stomachic, dyspepsia, colic, remittent fevers, bronchitis, dysentery of children and snakebite and as nerve tonic and insectifuge. Recent scientific studies have further revealed several other biological activities of *A. calamus* extracts or constituents. The plant has been found to show protective effect against nephrotoxicity, neuropathy and cardiomyopathy in experimental rats and also antifungal and antibacterial activities. The most important of the metabolites present in this medicinal plant are the flavonóides , lignin (Kawai *et al.*, 1999) polysaccharides and constituents of the essential oils, characteristic of the Thuja genus (Svajdlenka *et al.*, 1999), including diterpenes, monoterpenes (α-thujone, β-thujone, fenchone), and a sequiterpene. The polysaccharides as well as flavonoids (kercetin, campherol), tannins and proteins were identified by phytochemical investigations in ethanolic fraction of aerial part of *T. occidentalis* used in several pharmacological studies (Dubey and Batra, 2008).

The *Thuja occidentalis* antibacterial activity was observed against both gram negative and gram positive bacteria. The initiation of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study shows that antibacterial activity of 50 % methanolic extract of *Thuja occidentalis* against *B. subtilis* is best in 100 % concentration after 12 hrs (18.23±0.23 mm zone of inhibition). Although 75% concentration is having mild effect as 14.02±0.15 mm zone of inhibition. In Citrobacter 100% concentration of extract is having good antibacterial activity at maximum zone of inhibition 19.23±0.87 mm. On the other hand 75 % is showing static activity from, with zone of inhibition of 16.22±0.23 mm. For *E. coli* 100% concentration of extract show zone of inhibition 13.58±0.25 mm. Although the same effect of 75% concentration of extract is also revealing as showing zone of inhibition 12.74± 0.65 mm. In the case of Acetobacter 75% and 100% concentration of extract show good activity with zone of inhibition of 14.52±0.45 mm and 13.20±0.45 mm respectively. The above observations suggest that different concentration (50 %, 75 % & 100 %) were having good antibacterial activity against Acetobacter, Citrobacter, E. coli, B. subtilis, S. aureus and Enterococcus. Thus the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Norfloxacin and Ofloxacin. But extract is not potent than erythromycin and amoxicillin in these conditions (Table 1 and 2).

Another set of experiment the antifungal activity of *T. occidentalis* against different types of fungi. The best zone of inhibition against *Rhizopus orgae* in 100% and 50% concentration of extract is 12.3±0.032 and 12.3±0.033, in 75% concentration of extract that is 11.6±0.033 and 25% concentration is 9.6±0.06. For best zone of inhibition aganist *Ponicillium griseofulvm* in 100% concentration of extract is 7.0±0.057, in 75% and 25% concentration of extract is 06±0.057 & 06±0.087 mm and 50% concentration of extract is 5.0 ±0.057. *Alterneria alternate* 100% and 75% concentration of extract is having good antifungal activity at maximum inhibition of 10.6±0.0008 and 8.0±0.01. Although 50% zone of inhibition is 7.0 ±0.057 & 25% is having 6.0 ±0.057 concentration of extract. In *Fusiarium solani* 100% concentration shows good activity of zone of inhibition 05.6±0.033, 75% concentration is 4.6±0.033 and 50% 4.3±0.017 zone of inhibition and 25% constrictions of extract having 0 zone of inhibition (Table 3 and 4).

Thuja occidentalis leaves shows a high antibacterial activity against both gram negative and gram positive bacteria especially *Enterococcus faecalis* compared to other selected bacteria such as *Bacillus Licheniformis* From this above results proved that *thuja occidentalis* contain various phytochemical constituents like flavonoids, terpenoids, alkaloids, tannins, glycosides etc. due to flavanoid carotenoid content of the *Thuja occidentalis* extraction in ethanol has the lowest content of total carotenoid compared to methanolic extraction of *T. occidentalis* leaves. This methanol zone of inhibition observed against various bacterial strains like *Enterococcus faecalis.*

T. occidentalis can be considered as good sources of natural compounds with significant antioxidant and antimicrobial activity, and this can be attributed to the high percentage of polyphenolic compounds content. Therefore, with attention to results obtained, the present study provides a basis for the isolation and identification of compounds of biological interest from *T. occidentalis* for its potent activity.

The present investigation showed that stem and leaf extracts of *Thuja occidentalis* is quite effective against various pathogenic microorganisms. Therefore, it can be concluded that it may be used as natural antimicrobial agent to control the infection caused by the pathogens which otherwise becomes resistant to synthetic antibiotics.

5. SUMMARY

The present review reveals that the plant *Thuja occidentalis* is found to have therapeutic uses in treating various ailments. A detailed research work in the characterization and standardization is strongly required for this potential plant in developing its various formulations, which can ultimetly be beneficial for humans as well as animals. Further studies are warranted to explore much depth about this plant known by the name “The tree of life”. Their leaves contain essential oils used to treat fungus infections, cancer, moles and parasitic worms. The essential oil derived from the leaves is toxic. About the specie mentioned above, there are very ancient scientific records reflecting its traditional use by populations for the treatment of various symptoms, particularly respiratory infections of the upper tract and the common cold, to help with the treatment of bacterial infections, treatment of warts, Attributed primarily to its immunostimulant and antiviral properties. Thus, to elucidate the chemical composition of this species and its metabolites associated with these activities, many studies over time have given prominence to the constituents of the essential oil, which, in fact, have contributed to the pharmacological properties presented by *T. occidentalis*. However, a measure that certain majority monoterpenes were being disclosed as responsible also for the toxicity of the plant, preclinical studies latest revealed the presence of other metabolites of relevance, such as flavonoids, tannins and polysaccharides associated with various pharmacological activities, apart from those previously reported (anti-cancer, hypoglycemic, hepatoprotective, anti-ulcer, anti-diabetic and antioxidant). Over the years, clinical trials, particularly for the treatment of condyloma, confirm the efficacy of *T. occidentalis* for the treatment of lesions caused by HPV, but it is still necessary to establish more detailed methods for performing a more accurate assessment of efficacy and safety.

Another important point to be explored concerns the need to isolate the components responsible for the therapeutic activity of *T. occidentalis*, especially the constituents of EFTO whose preclinical studies indicate results of relevant pharmacological activities. Thus, the discovery of these markers can corroborate for standardization of herbal drugs, which allows initially set quality parameters for tinctures, extracts and other products obtained from *T. occidentalis*, some of which are already available on the market.

T. occidentalis has been widely used for many ailments in the traditions system like hepatoprotection, antioxidant, antidiabetic, anti microbial, anti cancer activity e.t.c because of its medicinal properties. In the experiment have suggested that different concentration of *T. occidentalis* extract (25%, 50%, 75%, & 100%) were having good antimicrobial activity against different types of bacteria and fungi. Thus the extract is showing varying activity against all micro organisms. On comparing the zone of inhibition of extract that standard antibiotics extract showed better activity than Norfloxacin and Ofloxacin. The plant may also further explored for its great verity of chemical constituent and wide range of bioactivities, as *T. occidentalis* is an endangered performance and efforts must be made to make its potent drug formulation for group exploit.

Medicinal herbs as potential source of therapeutics has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified. Once these local ethno medical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment. Determining the biological activity properties of plants used in traditional medicine is helpful to the rural communities and informal settlements. Several authors are currently being undertaken to isolate the active compounds by bioassay-guided fractionation from the species that showed high biological activity during screening. Therefore, these scientific investigations may be utilized to develop herbal drugs for these diseases and improved health status.

ACKNOWLEDGMENT

The authors are thankful to **Dr. K. Subramaniam**, Director General, Chhattisgarh Council of Science and Technology, Raipur (Chhattisgarh) India, for providing facility and technical support to carry out the above work.

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