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Research Article

ANTIMICROBIAL POTENCY AGAINST MICROBES FOUND IN CLINICAL SAMPLES AND TOXICITY STUDIES ON SELECTED MEDICINAL PLANTS

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ABSTRACT

The antibacterial and antifungal activities of *Terminalia arjuna, Tephrosia purpurea* and *Thuja occidentalis* extracts were investigated against the standard drugs, which are presently available in the market as Gentamicin, Cephradine, Amoxicillin clavulanate, Norfloxacin and Itraconazole. Well method was used for antimicrobial activity and the zones of inhibition were measured in millimeters. The antibacterial activity was observed on three gram negative bacteria *i.e. Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* and one gram positive bacteria *i.e. Staphylococcus aureus*. The antifungal activity was investigated against *Candida albicans* and *Aspergillus niger*. The cytotoxic effects (LD_{50}) of the crude extracts were also observed on brine shrimps (*Artemia salina*) using Etoposide as an internal standard. The crude extract of *T. arjuna* bark exhibited less potent antimicrobial activity as compared to crude extract of *T. arjuna* heart wood. Bark extract of *T. arjuna* showed no cytotoxic activity but heart wood extract showed this activity in brine shrimp bioassay technique. The crude extract of *T. purpurea* showed positive cytotoxic, potent antibacterial but no antifungal activity. The crude extract of *T. occidentalis* showed good antibacterial low antifungal and low cytotoxic activity. *T. arjuna* showed most potent antimicrobial and cytotoxic activity while *T. Purpurea and T. oocidentalis* posses less potent activities.

Key words: *Terminalia arjuna, Tephrosia purpurea, Thuja occidentalis,* Antimicrobial, Cytotoxicity.

INTRODUCTION

Terminalia arjuna, Tephrosia purpurea and *Thuja occidentalis* are the plants widely distributed in Asia. The study was carried out with the aim to investigate the hidden antimicrobial activity associated with anticancer activities in medicinal plants. *Terminalia arjuna* is commonly known as myrobalan belong to the family Combretaceae It is found through out the greater part of India. It contains flavonoids as arjunone, arjunolone along biacalein, luteolin, quercetin-7-O-rhamnoside, apigenin-7-O-neohesperidoside [1], triterpenoid saponins as arjunic acid, arjunolic acid, triterpene glycoside, triterpenoid, trihydroxytriterpene carboxylic acid, cardenolide, steroid, tannins [2] and other constituents. *T. arjuna* is used as cardiotonic [3], hypotensive [4]. Cheng and Lin (2002) determine antiherpes simplex virus type 2 activity of casuarenin from the bark of *Terminalia arjuna* Linn. Chouksey and Srivastava (2001) isolated new constituents from the roots of *Terminalia arjuna* that have antifungal action. Devi *et al*. (2008) worked on methanolic extract of *Terminalia arjuna* against Helicobacter pylori 26695 lipopolysaccharide-induced gastric ulcer in rats. It also has antianginal, hypolipidemic, hypocholestrolemic, antioxidant, anticancer, antimutagenic and diuretic effects. It is effective against congestive heart failure and myocardial infarction [5-8]. *Tephrosia purpurea* is commonly known as yellow thistle belongs to the family Leguminoceae. It is found in Southern Asia, Australia, Tropical America and Tropical Africa. It is annual or short lived perennial. It contains flavonoids, steroids, sterols, alkaloids, proteins, fixed oils and metal elements. *Tephrosia purpurea* is used as hepatoprotective, diuretic and anti-tumor (present in abdomen) agent. It is use in cough, asthma, bronchitis, bilious febrile attack, kidney disorders, liver insufficiency, infantile cirrhosis, boils, and pimples and bleeding piles. Roots and seeds possess insecticidal, pesticidal and hypoglycemic properties, it is also use in viral hepatitis [9-13]. *Thuja occidentalis* is commonly known as eastern white cedar belongs to the family Cupressaceae. It is an evergreen tree growing to 60 feet in height. *Thuja occidentalis* is employed in benign skin tumor, cancer, condylomata, neoplasms, papilomas, polyps and warts. Its major components are d-α-pinene, d-α-thujone, 1-fenchone, 1-borneol, acetic-, formic- and isovaleric-acids, terpineol, sabinene, camphene, camphor [14-16], valerianic acid, occidol-β-sitosterol, quercetin, rhodoxanthine, tannin, resins, mucilage and vitamin C etc. *T. occidentalis* is use for benign tumors, cancers, condylomata of penis and vulva, neoplasms, papillomas, plantar warts, polyps, tumors and warts. It is aphrodisiac, diaohoretic, diuretic, lactagogue and laxative. It is folk remedy for burns, colds, cough, debility, dysentery, dysmenorrhea, fever, gout, headache, inflammation, malaria, paralysis, rheumatism, swollen extremities, toothache and worms. It is antimutagenic, antimicrobial, antifungal and insecticidal [17-21].

The increase in candidiasis during the past years related to treatments such as antibiotic therapy, corticosteroid therapy, use of immunosuppressive drugs, radiotherapy, parental nutrition and antitumoral chemotherapy and the development of resistant strains have led to an increased interest in the development of new antifungal substances. Various herbal substances and essential oils exhibiting antifungal action have been tested. Studies are also performed to find out the antimicrobial and cytotoxic effect of herbal plant extracts having antitumour activity. The ever increasing resistance to human pathogens to current antimicrobial agent is a serious

medical problem resulting in the need for novel antibiotic prototypes. In the present work most common microbes found in clinical samples were utilized. The work was conducted to find out the chemotherapeutic potential and cytotoxicity of these plants and to determine their comparative antimicrobial potency.

MATERIALS AND METHODS

Preparation of Extract

The dried plant material of *T. arjuna* (bark and heart wood) and *T. purpurea* (aerial parts) was ground to powder while the fresh aerial parts of *T. occidentalis* was crushed in to small pieces and then soaked in ethanol at room temperature for 15 days. Then ethanolic extract was filtered. Filtrate was evaporated at reduce pressure using rotary evaporator (Buchi rota vapora E1-131) at 40 °C [22]. After evaporation of ethanol thick mass was obtained.

Antibacterial Assay

Antibacterial activity was carried out against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus*. The culture of organisms was maintained on stock culture agar and from the stock culture a loop full of the culture was inoculated in nutrient broth. The broth seeds were incubated at 37°C±1°C for 24 hours. Inocula were prepared by diluting 24 hours old culture in saline. A dilution of 1:100 was used in all the tests.

Modified soy agar Petri plates were prepared for testing the antibacterial activity of extracts. 0.1 ml of diluted culture was poured on each plate and the plates were dried for 30 minutes at 37°C. Wells of 6mm diameter were made with sterile cork borer in the inoculated agar. The wells were filled with the plant extract (0.1 g). Gentamicin, Cephradine, Amoxicillin clavulanate and Norfloxacin were used as standards in other wells separately. The plates were incubated for 24 hours at 37°C. At the end of incubation period the inhibition zones were measured [23].

Antifungal Assay

Antifungal activity was carried out against *Candida albicans* and *Aspergillus niger*. The culture of organisms was maintained on Saboraud dextrose agar and from Saboraud dextrose agar a loop full of culture was inoculated in Saboraud dextrose broth. The broth seeds were inoculated at 37°C±1°C for 24 hours. Inocula were prepared by diluting 24 hours old culture in saline. A dilution of 1:100 was used in the test.

The Petri plates of Saboraud dextrose agar were prepared and 0.1 ml of diluted culture was poured on each plate. The plates were dried for 30 minutes at 37°C. wells of 6mm diameter were cut with sterile cork borer in the inoculated agar. The wells were filled with plant extract (0.1g). Standard drug Itraconazole was used in next well. The plates were incubated for 24 hours at 37°C. At the end of incubation period the inhibition zones were measured. Each experiment was carried out at least three times along standard drugs [23].

Table 1: Antibacterial activity of plant extracts with standards

Plant Extract and Standard (100mg)	Bacteria with Zone of Inhibition (mm) and % Inhibition with Standard Drug			
	Escherichia coli	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Terminalia arjuna (Bark)	-	-	-	8(47.05%)
Terminalia arjuna (Heart wood)	7(46.66)	8(44.44)	10(-)	8(47.05%)
Tephrosia purpurea	7(46.66)	8(44.44)	10(-)	8(47.05%)
Thuja occidentalis	-	-	11(-)	8(47.05%)
Gentamicin	15	-	-	-
Cephradine	-	18	-	-
Amoxicillin clavulanate	-	-	-	-
Norfloxacin	-	-	-	17

Zone of inhibition is in mm; Parenthesis represents percentage of inhibition against standard

Table 2: Antifungal activity of plant extracts with standard

Plant Extract and Standard (100mg)	Fungi with Zone of Inhibition (mm)	
	Candida albicans	*Aspergillus niger*
Terminalia arjuna (Bark)	-	11
Terminalia arjuna (Heart wood)	9	11
Tephrosia purpurea	-	-
Thuja occidentalis	-	11
Itraconazole	-	-

Zone of inhibition ion mm; (-) No zone of inhibition

Table 3: Brine Shrimp Toxicity Bioassay of plant extracts

Dose (µg/ml)	**No. of shrimps**	**No. of survivors with *T.arjuna* (bark)**	**LD_{50} (µg/ml)**	**No. of survivors with *T.arjuna* (heart wood)**	**LD_{50} (µg/ml)**	**No. of survivors with *T.purpurea***	**LD_{50} (µg/ml)**	**No. of survivors with *T.occidentalis***	**LD_{50} (µg/ml)**	**Std. Drug**	**LD_{50} (µg/ml)**
1000	30	28		12		12		18			
100	30	28	_	20	435.4451	16	330.6530	26	_	Etoposide	7.4625
10	30	30		28		30		30			

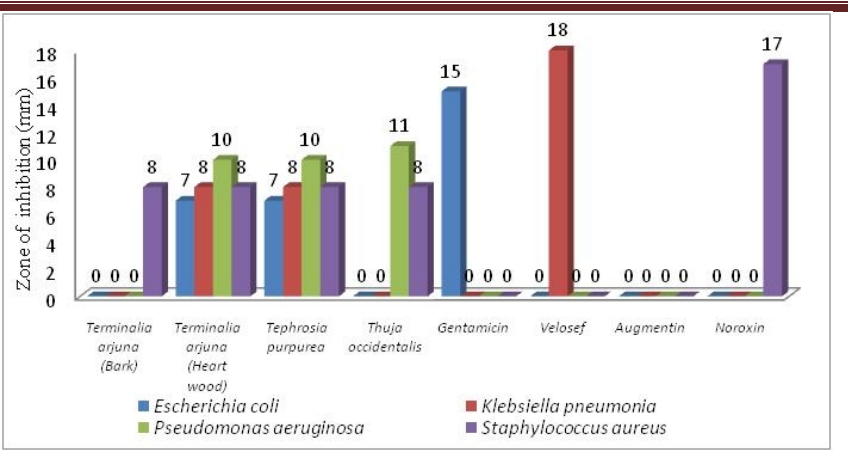

Figure 1: Antibacterial Activity of *T. arjuna* (Bark, Heart wood), *T. purpurea* and *T. occidentalis* Ethanolic Extracts

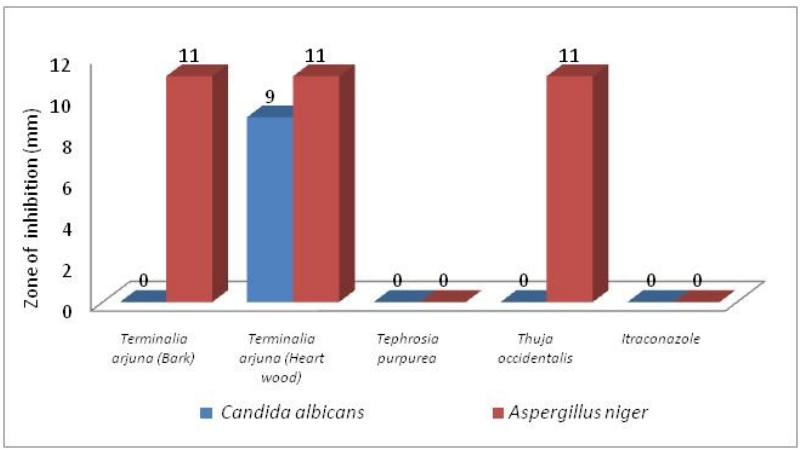

Figure 2: Antifungal Activity of *T. arjuna* (Bark, Heart wood), *T. purpurea* and *T. occidentalis* Ethanolic Extracts

Brine Shrimp Bioassay (LD_{50})

The three crude plant extracts were used for the estimation of LD_{50} activity in brine shrimps bioassay. The procedure described by Mayer was adopted for this work [24]. Samples of three different concentrations 10, 100 and 1000 µg/ml were prepared. Brine Shrimp (*Artemia salina*) nauplii were hatched in a specific tank. 30 shrimps were added in each sample vial and then sea water was added to make the volume 5ml. later on dry yeast suspension was added as food to each vial including control. The vials were kept for 24 hours, thereafter the active nauplii were counted and death percentage was calculated at each dose and analyzed the data with Finney computer programme in order to determine LD_{50} values.

RESULTS

The antimicrobial and cytotoxic activity of crude extracts of *T. arjuna* bark, *T. arjuna* heart wood, *T. purpurea* and *T. occidentalis* were investigated to find out the antimicrobial & cytotoxic activities present in these chemotherapeutic agents. The antibacterial activity was observed on three gram negative bacteria *i.e. Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa* and one gram positive bacteria *i.e. Staphylococcus aureus* using Gentamicin, Cephradine, Amoxicillin clavulanate, and Norfloxacin as standards.

The antifungal activity was investigated against *Candida albicans* and *Aspergillus niger*. Itraconazole (1mg) was used as standard. Fungi were found resistant against standard drug. The extract of *T. arjuna* bark showed no antibacterial activity against *E. coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* while it showed antibacterial activity (8mm) against *Staphylococcus aureus* (Fig.1). The *T. arjuna* extract exhibited no antifungal activity against *Candida albicans* while against *Aspergillus niger* it show antifungal activity (11mm). The extract of *T. arjuna* heart wood show antibacterial activity against *E. coli* (7mm), *Klebsiella pneumoniae* (8mm), *Pseudomonas aeruginosa* (10mm) and *Staphylococcus aureus* (8mm). The extract of *Terminalia arjuna* heart wood also displayed antifungal activity against *Candida albicans* (9mm) and *Aspergillus niger* (11mm) (Fig. 1,2). The extract of *T. purpurea* showed antibacterial activity against *E. coli* (7mm), *Klebsiella pneumonia* (8mm), *Pseudomonas aeruginosa* (10mm) and *Staphylococcus aureus* (8mm) while it exhibited no antifungal activity against *Candida albicans* and *Aspergillus niger* (Fig. 1,2). The extract of *T. occidentalis* showed antibacterial activity against *Pseudomonas aeruginosa* (11mm) and *Staphylococcus aureus* (8mm) and it displayed (11mm) zone of inhibition against *Aspergillus niger*.

The cytotoxic activity of crude extracts was checked on brine shrimp (*Artemia salina*) using Etoposide as standard. The extracts were used in three concentrations 10, 100 and 1000 µg/ml. *T. arjuna* bark extract and *T. occidentalis* show no LD50 while *T. arjuna* heartwood extract (433.4451µg/ml) and *T. purpurea* (330.6530µg/ml) were found cytotoxic (Table 3).

DISCUSSION

The findings of this research work suggest that *T. arjuna* heart wood had most potent antibacterial and antifungal activity as compared to *T. arjuna* bark, *T. purpurea* and *T. occidentalis* crude extracts (Table 1-2). Chouksey and Srivastava in 2001 and Devi *et al*., 2008 also reported antifungal and antihelicobactor activity of *T. arjuna*. Kuo *et al*. in 2005 found that casuarinin from the bark of *T. arjuna* induces apoptosis and cell cycle arrest in human breast adenocarcinoma [8]. Antiherpes simplex virus type 2 activity of casuarenin from the bark of *Terminalia arjuna* Linn. was found by Cheng and Lin in 2002 [5]. *T. arjuna* heart wood was found cytotoxically active while bark is cytotoxically inactive. *T. purpurea* is also cytotoxically active while *T. occidentalis* is cytotoxic at higher concentrations (Table 3). *T. arjuna* bark extract showed antibacterial activity against *Staphylococcus aureus* (47.05%). It showed antifungal activity against *Aspergillus niger*. *T. arjuna* haeartwood showed antibacterial and antifungal activity against all bacteria and fungi. Most potent antibacterial activity was against *Pseudomonas aeruginosa* while most potent antibacterial activity was against *Aspergillus niger*. Among all these plant extracts the spectrum of antimicrobial activity of *T. arjuna* heartwood extract was found highest. The extract of *T. purpurea* showed antibacterial activity against *E. coli, Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Against *Pseudomonas aeruginosa* it give antibacterial activity. Gulecha and Sivakuma in 2011 also analysed anticancer potential of *Tephrosia purpurea* and *Ficus religiosa* using MCF 7 cell lines [13]. In presenr work extract of *T. purpurea* showed no antifungal activity. The extract of *T. occidentalis* showed antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* It produced inhibition against *Pseudomonas aeruginosa* and *Aspergillus niger*. Delespaul *et al*. in 2000 also determined antifungal activity of essential oils of *Thuja* by different screening methods [20]. Biswas *et al*. in 2011 demonstrated thujone-rich fraction of *Thuja occidentalis* as major anti-cancer potentials containing fraction by in vitro studies on A375 cells [17].

Cytotoxic activity of *T. purpurea* is found most potent (330.6530%) then crude extract of *T. arjuna* heart wood (435.4451%) and *T. occidentalis* that show activity at high concentrations. The crude extract of *T. arjuna* bark is found cytotoxically inactive.

CONCLUSION

From the above investigation it can be concluded that the chemotherapeutic agents, *T. arjuna* displayed most effective activity in antimicrobial and cytotoxic experiments while *T. purpurea* and *T. occidentalis* exhibit weak activities as compared to *T. arjuna*. This relation may be due to volatile constituents present in all these chemotherapeutic agents as from previous literature it was find out that the plants having volatile constituents also have antimicrobial property.

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