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# ANTIMICROBIAL POTENTIAL OF SOME INDIAN MEDICINAL PLANTS AGAINST DRUG-RESISTANT PATHOGENS

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**ABSTRACT:** Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. Traditional system of medicine is found to have utilities as many accounts. Due to population rise adequate supply of drug and high cost of treatment in side effect along with drug resistance has been encountered in synthetic drugs, which has lead to an elevated emphasis for the use of plants to treat human diseases. A major part of the total population in developing countries still uses traditional folk medicine for different diseases obtained from plant resources. Due to the indiscriminate use of antimicrobial drugs, the emergency of human pathogenic microorganism's resistance has been increased. According to WHO, as many as 80% of world's population living in rural areas rely on herbal traditional medicines as their primary healthcare, the properties and uses of medicinal plants are interestingly growing. Thus, due to prevalence of microbial resistance in today's era and search for new antimicrobial agents, the aim of the study was localized on the screening of solvent extracts of some Indian medicinal plants viz. *Terminallia arjuna, Withania somnifera, Emblica officinalis, Ocimum gratissimum* and *Trigonella foenum graecum* prepared in methanol and petroleum ether against drug resistant pathogens. The results confirmed the antimicrobial potential of plants against the pathogens studied at concentration 100 mg/ml.

**INTRODUCTION:** Pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases and also more toxic for human being during long term therapy [1, 2] so there is a need for less toxic, more potent and non anti-infectives antibiotics. Therefore the use of plant extracts and phytochemicals with known antibacterial properties may be of immense importance in therapeutic [3].

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Most plants are medicinally useful in treating disease in the body and in most of cases the antimicrobial efficacy value attributed to some plants is beyond belief. Conservative estimates suggest that about 10% of all flowering plants on earth have at one time, been used by local communities throughout the world but only 1% have gained recognition by modern scientists[4].

*Withania somnifera* (family Solanaceae) is a medicinally important herb used in number of Indian herbal formulations. In India, it is locally known as 'Ashwagandha' and is considered as *Indian Ginseng*. Roots of the plant are major source of active chemical substances and are traditionally used to cure ulcers, fever, cough, dyspnoea, consumption, dropsy, impotence, rheumatism, toxicosis and leucoderma[5]. The plant had been reported to grow in wild and is also cultivated in

selective areas of India. Their pharmacological properties are diverse ranging from anti-inflammatory, anti-tumor, anti-stress, anti-oxidant, immunomodulatory, hemopoetic and cardio-protective effects [6]. Amla is a component of Triphala and an important rasayana called Chyawanprash in ayurvedic medicine[7]. *Emblica officinalis* famously known for its hepatoprotective and antioxidant activities [8, 9].

Amla extract posses anticancer, antisclerotic, lipid lowering, hepatoprotective, anti- HIV activitiesv[10] and inhibits thioacetamide-induced oxidative stress and hyper proliferation in rat liver [11]. The alcoholic and aqueous extract of amla has powerful retarding effect on ochratoxin haemolysis on RBC[12]. The wide use of *Emblica officinalis* fruits for various purposes prompted us to select for screening of antibacterial activity. *Ocimum sanctum* L. is held sacred by Hindus and is used as medicinal plants in day to day practice in Indian homes for various ailments.

In ayurveda tulsi has been well documented for its therapeutic potentials and described as Dashemani, Shwasaharni (anti-asthmatic) and anti-kaphic drugs (kaphaghna). Several medicinal properties have been attributed to *Ocimum sanctum* L. different parts of Tulsi plant e.g. leaves, flowers, stem, root, seeds etc. are known to possess therapeutic potentials and have been used, by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, anti-diabetic, anti-fertility, hepatoprotective, hypotensive, hypolipidmic, anti-microbial, antifungal activity against *Asperigillus niger*. *Terminalia arjuna*, a deciduous tree belonging to Combretaceae family, is of 20-30 m height and is found ubiquitously in Bangladesh and India.

It has been documented that bark extract from *Terminalia arjuna*, contains following compounds: acids such as arjundic acid, terrninic acid, glycosides-argentine arjunosides I-IV, strong antioxidants such as, flavones, tannins, oligomeric proanthocyanidins and minerals[13]. Arjuna bark (*Terminallia arjuna*) is thought to be beneficial for the heart. Powdered extract of the above drug provided very good results to the people suffering from Coronary heart diseases[14, 15]. *T. foenum-graecum* (fenugreek) is an important spice in India, Egypt, Saudi Arabia, Iran, Armenia and Turkey. *Trigonella* seeds are bitter, mucilaginous, aromatic, tonic, emollient and anti-carcinogenic agents[16]. Antimicrobial, antioxidant and anti-inflammatory properties of the plants of Uttarakhand State were investigated[17-20].

## MATERIALS AND METHODS:

**Collection of plant materials:** The plant materials were collected from Dehradun (U.K) and hill regions of Jammu & Kashmir (J&K), India.

**Preparation of plant extracts:** The method for preparation of plant extracts was modified [21]. Thoroughly washed dried leaves of five plants viz. *Terminallia arjuna, Withania somnifera, Emblica officinalis, Ocimum gratissimum,* Trigonella foenum graecum were dried under shade for one week. Plant parts were placed in an oven at 38 °C and then powdered with mortar and pestle. The powdered material of each of these plants in quantity, 1 gram/100 ml was soaked separately in petroleum ether and ethanol for 72 h. Mixture was stirred after every 24h using a sterile glass rod. At the end of extraction, extract was passed through whatmann filter paper no. 1. The filtrates obtained were concentrated in vacuum using rotary evaporator at 30$^{o}$C.

**Culture Media:** The media used for antibacterial test was nutrient agar/ broth and Sabourauds dextrose agar/ broth for antifungal test.

**Microorganisms used:** The test organisms *Salmonella abony, Escherichia coli,* two strains of Methicillin-resistant *Staphylococcus aureus, M. luteus* and *L. plantarum* along with the fungal test organisms *Candida albicans, Aspergillus niger* were procured from National Chemical Laboratory Pune, India.

**Determination of antibacterial and antifungal activity by agar well diffusion Method:**

The petroleum ether and ethanol extracts of plants were tested for antibacterial and antifungal activity using agar well diffusion method at sample concentration 1g/100ml. The agar well diffusion method was modified [22]. Nutrient agar medium (NAM) was used for bacterial cultures. The culture medium was inoculated with the bacteria separately suspended in nutrient broth. Sabouraud's dextrose agar/broth was used for fungal cultures. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar

and filled with plant extracts and solvent blanks. Solvents, petroleum ether and ethanol were used as negative controls. Standard antibiotic (Erythromycin, 1 mg/ml) was simultaneously used as the positive control. The plates were then incubated at 37 $^0$C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed.

For assaying, antifungal activity of plant extracts, Sabouraud's dextrose agar/ broth medium plates were used. The same procedure as that for determination of antibacterial property was adopted and then after the diameter of zone of inhibition was observed after 48-72 h. Fucanazole (1mg/ml) was used as standard for determination of antifungal activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

**Determination of Minimum Inhibitory Concentration (MIC):** MIC value of potent plant extracts was determined by the method with some modifications [23]. Plant extract was prepared in highest concentration (200 µg/ml) in sterile distilled water and is serially diluted with N-saline (0.85 % NaCl) and similar quantity of bacterial suspension was added to different test tubes and incubated for 48 h. The inhibition of turbidity appeared in the minimum dose at which total growth of bacteria gets killed is known as minimum lethal concentration (MLC) while little turbidity appeared in the minimum amount of dose of plant extract which inhibits the growth of bacteria is known as Minimum Inhibitory Concentration (MIC).

## RESULTS AND DISCUSSION:

The present study reveals the potent antimicrobial spectrum of ethanolic extracts of *Oscimum sanctum* and *Withania somnifera* against the microbial cultures studied. The petroleum ether extracts of *Ocimum sanctum, T. arjuna* and *E. offficinalis* and *T. faenum graceum* showed significant antimicrobial activity but less potential than that of the ethanolic extracts. In the present investigation, it was found that both petroleum ether and ethanol extract of the plant studied have potent antibacterial activity. It was found that petroleum ether extracts of Tulsi, *Terminalia arjuna, E. officinalis* have significant antibacterial activity against *S. aureus* (MRSA-isolated from pus). Amongst all these extracts, it was found that petroleum ether extracts of *E. offficinalis* had potent activity against MRSA (isolated from pus). Simultaneously it was found that ethanolic and petroleum ether extract of tulsi had potent activity against MRSA (isolated from blood). The results showed that ethanolic extracts of tulsi and w. somnifera had significant antibacterial activity organist MRSA (isolated from pus). Amongst these both extracts of *W. somnifera* had potent activity in comparison to Tulsa ethanol extract against MRSA (isolated from pus). It was found that *E.coli* is the most sensitive pathogen against each and every solvent while MRSA (isolated from blood) was found to b most resistant pathogen.

Both the petroleum ether and ethanolic extract of the specific plant are antifungal in nature. Tulsi petroleum extract showed potent antifungal activity against *C. albicans* in comparision to tusi petroleum ether extract. *T. fenum gracemum* petroleum ether extract showed potent antifungal activity against *C. albicans* while its extract showed no activity against *A. niger*. *E. officinalis* petroleum ether extract showed potency against *C. albicans* while no activity was showed against A. niger. *W.somnifera* ethanolic extract showed maximum potency against *A.niger* in comparision to *C.albicans*. *W. somnifera* and *T. arjuna* petroleum ether extracts showed no activities against any of the fungal culture. Tulsi ethanolic extract was found to be more active against *C. albicans* in comparision to *A. niger*.

The results are shown in **Table 1** and **Figure 1**. The MIC and MLC values of the potent extracts are shown in **Table 2 (a&b)**.

| | | *E. coli* | *L. plantarum* | *S. abony* | *M. Luteus* | *S. aurues (Blood)* | *S.aurcus (pus)* | *A. niger* | *C. albicans* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Ocimum Sanctum (Petroleum Ether)* | 28.0 | 12.0 | 18.0 | 15.0 | 15.0 | 12.0 | NA | 28.0 |
| 2 | *Ocimum Sanctum (Ethanol)* | 25.0 | 8.0 | 12.0 | 15.0 | 12.0 | 10.0 | 17.0 | 18.0 |
| 3 | *T. Arjuna (Petroleum Ether)* | 12.0 | 10.0 | NA | NA | 12.0 | NA | NA | NA |
| 4 | *W. Samnifera (Petroleum Ether)* | 15.0 | NA | 10.0 | NA | NA | NA | NA | NA |
| 5 | *W. Samnifera (Ethanol)* | 20.0 | NA | 17.0 | 18.0 | 18.0 | NA | 21.0 | 16.0 |
| 6 | *E. offficinalis (Petroleum Ether)* | 18.0 | 18.0 | NA | 15.0 | 25.0 | NA | NA | 17.0 |
| 7 | *T. Faenum graceum (Petroleum Ether)* | 15.0 | 12.0 | NA | NA | NA | NA | NA | 20.0 |

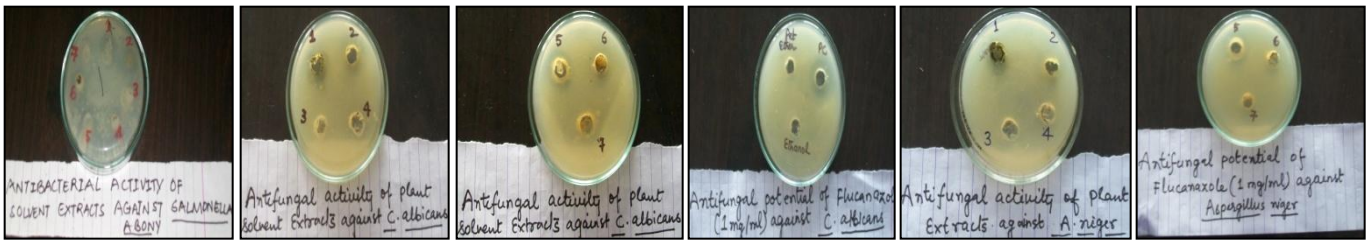


**FIGURE 1: ANTIMICROBIAL ACTIVITY OF THE SOLVENT EXTRACTS OF THE PLANTS**

**TABLE 2 (A): MIC / MLC OF PLANT EXTRACTS AGAINST BACTERIAL PATHOGENS**

| Extract | Pathogen | MIC (µg/ml) | MLC (µg/ml) |
|---|---|---|---|
| *W. Somnifera* (Ethanol) | *Micrococcus* | 0.01 | 0.1 |
| *W. Somnifera* (Ethanol) | *E.Coli* | 1 | 10 |
| *W. Somnifera* (Ethanol) | *MRSA (pus)* | 0.1 | 1 |
| *Emblica Officinalis* (Petroleum Ether) | *E. coli* | 0.01 | 0.1 |
| *Emblica Officinalis* (Petroleum Ether) | *L. plantarum* | 0.01 | 0.1 |
| *Emblica Officinalis* (Petroleum Ether) | *MRSA (pus)* | 0.1 | 1 |
| *Ocimum Sanctum* (Petroleum Ether) | *S. abony* | 1 | 10 |
| *Ocimum Sanctum* (Petroleum Ether) | *E. coli* | 0.01 | 0.1 |
| Osimum Sanctum (Ethanol) | E. coli | 0.01 | 0.1 |

**TABLE 2 (B): MIC / MLC OF PLANT EXTRACTS AGAINST FUNGAL PATHOGENS**

| Extract | Pathogen | MIC (µg/ml) | MFC (µg/ml) |
|---|---|---|---|
| *Ocimum sanctum* (Petroleum Ether) | *C. albicans* | 0.01 | 0.1 |
| *Ocimum sanctum* (Ethanol) | *C. albicans* | 0.01 | 0.1 |
| *Trigonella – Foenum – graceum* (Petroleum Ether) | *C. albicans* | 1 | 10 |
| *W. somnifera* (Ethanol) | *A. niger* | 0.1 | 1 |

**CONCLUSION:** Research in medicinal plants has gained a renewed focus recently. The prime reason is that other system of medicine although effective come with the number of side effects that leads to complications. In the above investigation different solvent extracts of medicinal plants shown to have anti- microbial activity against various drug resistant pathogens. *E. officinalis* have significant antibacterial activity against *S. aureus* (MRSA-isolated from pus). Further investigations may lead to development of new antibiotic (s) of high potency.

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## REFERENCES:


1. Davis: Inactivation of the antibiotics and the dissemination of resistance genes. Science1994; 264: 375-382.
2. Qadrie LZ, Jacob B, Anandan R, Rajkapoor B,Ulla RM): Antibacterial activity of ethanolic extracts of indoneesiell echioides(L)Nees evaluated by the filter paper disc method.*pak.j.pharm.sci 2009*,22:123-125
3. Olukoya DK ,Idika N.odugbemi T: Antibacterial activity of some medicinal plants from Nigeria *j. Ethnopharmacol 1993*;39:69-72
4. Ahmadiani A, Javan M, Semnanian S, Barat E and Kamalineyad M :Antiinflammatory and antipyretic effects of *Trigonella foenum graecum* leaf extracts in rats. *Journal of Ethnopharmacology*, 2001; 75: 283–286.
5. Devi U, Parastoo KA, Parisa KA and Devindra S: *Ind. J Pharm Sci, 2012* 34: 927-932.
6. Singh S, Tanwer BS and Khan M,) *Advances in Applied Science Research 2011; 2* (3): 47-52.
7. Kaur S, Arora S, Kaura K: The in-vitro antimutagenic activity of Triphlaan Indian herbal drug *Food Chem Toxicol* 2002; 40:527-534.
8. Jose JK, Kuttan R: Hepatoprotective activity of *Emblica officinalis* and Chavanaprash *J Ethnopharmacol*, 2000; 72: 135-140.
9. Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK: Antioxidant activity of tannoi*d*priniciples of *Emblica officinalis* (Amla*). Indian J Exp Biol* 1999; 37: 676-80.
10. Khan MT, Lampronti I, Martello D, Bianchi N, Jabbar, Choudhuri MS, Datta BK, Gambari R): Identification of pyrogallol as an antiproliferative compound present in extracts from the medicinal plant *Emblica officinalis*; effects on *in vitro* cell growth of human tumor cell lines. *Int J Oncol* 2002; 21(1): 187-192.
11. Sultana S, Ahmed S, Sharma S, Jahangir T: *Emblica officinalis* reverses thioacetamide-induced oxidation stress and early promotional events of primary hepatocarcinogenesis J Pharmacol 2004; 56(12): 1573-1576.
12. Verma RJ, Chakraborthy D: Protection from oxidative damageusing *Emblica officinalis* Gaertn extracts in case of ochratoxin induced toxicity in normal human RBC s*Natural Product Radiance* 2006;6(4): 310-314.
13. Brand-Williams W, Cuvelier ME & Berset C: Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology 1995;* 28: 25-30.
14. Maulik SK: Focused Conference Group: P16 - Natural products: Past and future? Role of *Terminalia arjuna* an Indian medicinal plant in cardiovascular diseases. *Basic and Clinical Pharmacology and Toxicology* 2010; 107(1): 445-446.
15. Shukla SK, Dwivedi S, and Singh SB & Sharma UR: *Terminalia arjuna* as a therapeutic and preventive modulator in experimentally induced myocardial infarction. *Diabetes and Vascular Disease Research* 2011; 8(1): 80-81.
16. Devasena T and Menon VP: Fenugreeek affects the activity of b-glucoronidase and mucinase in the colon. *Phytotherapy Research* 2003; 17: 1088–1091.
17. Hafeez B, Haque R, Parvez S, Pandey S, Sayeed I and Raisuddin S: Immunomodulatory effect of fenugreek (*Trigonella foenum graecum* L.) extract in mice. *International Immunopharmacology* 2003; 257–265.
18. Harve G and Kamath V: Larvicidal activity of plant extracts used alone and in combination with non synthetic larvicidal agents against *Aedes aegypti*. Indian Journal of Experimental Biology 2004 ; 42: 1216-1219.
19. Hannan JM, Ali L, Rokeya B, Khaleque J, Akhter M, Flatt PR and Abdel-Wahab YH : Soluble dietary fibre fraction of *Trigonella foenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. *British Journal of Nutrition* 2007; 97: 514–521.
20. Kaviarasan S and Anuradha CV: Fenugreek seed polyphenols protect liver from alcohol toxicity: a role on hepatic detoxification system and apoptosis. *Pharmazie, 2007;* 62: 299–304.
21. Alade PI, Irobi ON: Antimicrobial activities of crude leaf extracts of *Acalypha wilkensiana*. *Journal of Ethnopharmacology*1993; 39: 171-174.
22. Perez C, Anesini C: *In vitro* antimicrobial activity of Argentine folk medicinal plants against *Salmonella typhii*. Journal of Ethnopharmacology1993; 44: 41-46.
23. Vollekova AD, Sochorova R: Isoquinoline Alkaloids from *Mahonia aquifolium* stem bark is active against Malassezia sp. Folia Microbiol., 2001 46: 107-111.


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