IN VITRO ANTI MICROBIAL POTENTIAL OF ARTEMISIA ROXBURGHIANA BASSER

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ABSTRACT

In traditional medicine, herbal remedies are often used for treating burns, dermatophytes, and other infectious illnesses. Based on ethnopharmacological and taxonomic data, we tested the antibacterial activities of aqueous and ethanolic extracts of selected medicinal plants in vitro using the agar diffusion-method against selected human pathogenic bacteria. The antimicrobial properties of the leaves of five different plants were tested. Alternative medical practices have long made use of several plant species from all over the globe. There was an extraction process including water and methanol for the powdered leaves of each plant. To dry out the solvent extracts and concentrate them, a rotary flash evaporator was utilised. The dried residue's bactericidal effectiveness was measured by dissolving it in ethanol (1:10 w/v). Various bacteria, including Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis, and Yersinia enterocolitica, were employed for in vitro antibacterial screening. Methanol extracts demonstrated more diverse effects on these species than water extracts, indicating that the active components may be present in methanol extracts of all the plants we studied. Many different types of ailments, including as those affecting the immune system, nervous system, digestive tract, respiratory system, skin, and even a high temperature, have been proven to respond well to traditional herbal treatment.

Key words: Antimicrobial, Antioxidant, Medicinal plants, Human pathogens

INTRODUCTION

1.1 MICROBES

Microbes are the tiniest creature that cannot be seen by necked eyes. Microorganisms like fungi, algae, bacteria, and viruses. These are unicellular organism that grows rapidly under moisture as well as in warm place. The two primary categories of bacteria are gram-positive ones like Staphylococcus aurous and gram-negative bacteria like *E. coli*. Some specific types of bacteria are

pathogenic E.g. which cause cross infection. Complex mold and fungi organisms have a slow growth rate (**McArthur, E.D *et al* 1978**).[1]

According to the World Health Organization, almost 80% of the world's population relies on herbal and traditional medicine for their main healthcare requirements (WHO). In Asia, the use of herbal medicine also represents a long history of human interaction with the environment. In the treatment of infectious diseases, we can use traditional medicine which also contained a varied range of ingredients from a plant (**Hayat, M.Q *et al* 2010)[2].**

1.1 INFECTION

Infection involves interaction between infecting microorganisms and animal bodies. Microbes known as pathogens can infect a host and cause disease. Infections are classified into various types 1) initial infection. 2) Subsequent infection. 3) Focal infection.When infection at localized sites then the appendix or tonsil generalized effect is produced. Cross infection occurs when a patient already has a disease and a new infection is set up from another source. (**Stanton, D.*et al* 2002).[7]**

1.2 ANTIMICROBIAL AGENT

The treatment of infection is based upon non- specific antimicrobial antifungal agents done by an antimicrobial agent which is often strong irritants as well being toxic to the patient. Herbal medicine and traditional medicine are both considered to rapidly growing health systems. It is increasing greatly in developed countries (WHO 2002). Worldwide it remains widespread in developing countries. The applications of the traditional medicinal plant have been employed as remedies long before the development of western medicine with the advent of the science of technology. (**Shinwari, M.I. *et al* 2000**).

1.3 ANTIMICROBIAL HERBAL DRUG

As per WHO '80% population of the world depend on herbal medicine for their primary health care needs". Antimicrobial activities are known to after protection against various bacteria with medicinal plant viral and other diseases and also find the industrial application (**Tobe, K *et al*. 2006**).[11]

Antibiotic resistance leads to access health care value & pull effort to examine novel agents from natural sources offer clues to discovered new antimicrobial agents & antibiotic resistance also serious health problem with significant mortality & morbidity from treatment failure the activity of antibacterial & antifungal of a plant is beneficial to food and dairy industries also effected method of antimicrobial contamination for the treatment of various disease. The Indian plants are used because of plant constituent rich untapped cure medicinal plant due to various plant parts and a tropical climate like seeds, bark, stem, fruit & root. An excellent source for discovering new antimicrobial medicines is plant extract for antibacterial activity. (**Enserink, M *et al*2008).[12]**

Table 1.1 List of Antimicrobial plants

S. NO	Plant name	Part used	Extract	concentration	References
1	*Allium sativa (*Amaryllidaceae)	Fruit	Ethanol	80-100mg/ml	*Atheer* 2014
2	*Justica adhatoda* (Acanthaceae)	Leaf	Chloroform	20mg/ml	*Jaypriya et al*.,2015
3	*Azadiracta indica* (Meliaceae)	Leaf	Ethanol	0.5mg/ml	*Hala et al*., 2015
4	*Moringa oleifera* (Moringaceae)	Leaf	Acetone, water	5mg/ml	*Saranrajet al*.,2014
5	*Piper betle* (Piperaceae)	Leaf	Ethanol	50-100mg/ml	*Saranrajet al*., 2014
6	*Tinospora cardifolia* (Menispermaceae)	Stem	Ethanol	20mg/ml	*Priyanka et al.,* 2014
7	*Acacia nlotica* (Leguminosae)	Leaf	Ethanol and chloroform extract	50mg/ml	*Amjadet al*.,2014

1.4 ANTIOXIDANT

Antioxidants are essential substances for all animals and plants. Oxidation is a reaction of a chemical molecule that transfers electrons from a substance to an oxidizing agent. After that free radicals are produced during the oxidation reaction. Antioxidants protect cells from damage. Oxidants have the capability of preventing the oxidation of another molecule (**Sah, S *et al* 2006) [12].**

1.4.1 Types of antioxidants

- **Endogenous antioxidant**

Endogenous antioxidants are essential enzymes that catalytically remove oxidants that are superoxide dismutase, superoxide reductase, catalase, etc (**Shah, G.C *et al.2008*) [28]**

List of Antioxidant plants

S.no.	Plant name	Part used	Extract	Concentration	Reference
1	*Curcuma domestica*	Rhizome	Ethanol	100mg/ml	*Corina et al*.,2015
2	*Dascus carota*	Leaf & seed	Methanol	80 mg/ml	*Ksouri et al*., 2014
3	*Emblica officinalis* (Euphorbiaceae)	Seed	Methanol	20mg/ml	*Priya et al*., 2012
4	*Glycyrrhiza glabra* (Leguminaceae)	Rhizome & root	Methanol	0.02-0.08mg/ml	*Rathnavel et al.*, 2014
5	*Morinda cirtifolia* (Rubiaceae)	Fruit	Chloroform, water, and ethanol	85.8mg/ml	*Vennilaet al*.,2014
6	*Ocimum basilicum* (Lamiaceae)	Leaf	Ethanol	0.06mg/ml	*Vardapetyanet al*.,2014
7	*Santalum album* (Santalaceae)	Wood	methanol	100 mg/ml	*Heenaet al*., 2014

1.5 Phytochemical Screening (WHO 2009) [21]

The chemical composition of the plant parts was evaluated by preparing and testing extracts in both methanol and water. Extraction solvents including water and methanol, as well as powdered samples, were put through standard chemical analyses to identify the substances contained inside.

Test for carbohydrates

Molish test: Each of the three different sulphuric acid solutions was added to the test tube in equal volumes, and when the liquids were mixed, the mixture became crimson. The presence of carbohydrates may be detected in this photograph.

Fehling test: Both Fehling A and Fehling B solutions were poured into the sample powder and put in a water bath for a suitable amount of time. The brick's red color may be seen here. There is evidence of carbohydrates.

Benedict's test: Adding 8 drops of Benedict's reagents to the powdered sample and shaking vigorously for 5 minutes will get the following results. Carbohydrate is represented in this way.

Test for alkaloids: Using a drop or two of hydrochloric acid and a filter, a little amount of dried powder (sample) was treated. Alkaloid agents were used to testing the filtration.

Mayer's reagents: Add a modest amount of Mayer's reagent to the aforesaid filter to generate a cream precipitate. The presence of alkaloids may be seen here.

Dragendorffs reagents: Add a modest quantity of Dragendorff's reagents to the aforesaid filter and you get an orange-brown precipitate. Alkaloids are visible in this image.

Test for flavonoids: Dilute ammonia solution should be added to the plant extract filter, followed by concentrated sulphuric acid. Yellow is the color it takes on. Flavonoids were detected in the extract.

Test for steroids and triterpenoids:
Salkowaski test: Red color at the lower layers indicate the presence of steroid and Yellow color at the lower layers indicates the presence of triterpenoids.

Libbbermann burchard test: If you want to determine whether or not steroids or triterpenoids are present, check for a brown ring to form at the interface of the two layers, for the top layer's color to shift to green, or for a deep red color to arise. All of these things are telltale signs of their existence.

Test for tannins: Plant extracts are subjected to the vanillin hydrochloric acid reagent to extract the vanillin hydrochloride. Due to the development of phloroglucinol, which indicates the presence of tannins, it takes on a pink or crimson hue.

Test for protein:
Mellon's reagents: Mercuric nitrate in the presence of nitric acid and nitrous acid (Mellon's reagents) typically results in a white precipitate that becomes crimson when heated.

Ninhydrin Test: In a separate container, add 2 drops of freshly-produced 0.2 percent Nihydrine reagent after heating the extract. As peptides and amino acids become blue in color, this may indicate their existence (PROTEIN).

Test for glycosides:

Keller-killani test: Small quantities of ferric chloride were added to the surface of the concentrated sulphuric acid, and the ferric chloride was heated to dissolve the acetic acid in the conc sulphuric acid. The reddish brown color at the junction shows the presence of cardiac glycosides, which progressively turn blue.

Test for saponins:

Foam test: In a graduated cylinder, one milliliter of the extract solution is agitated for 15 minutes at a concentration of 20 milliliters in distilled water. An inch-thick coating of foam is a sign that saponins are present.

1.6 CHROMATOGRAPHIC STUDIES (WHO 2009) [14]

Definition:

The differential affinity of solutes between two immiscible phases is used to separate molecular mixtures in chromatography. The stationary phase consists of a huge fixed surface area bed, whereas the mobile phase consists of a fluid moving through or over the stationary phase's surface.

1.5.1 Thin layer chromatography

Preparation of TLC Plate: washed the TLC plates and dried them in the oven.TLC plates are prepared by the pouring method. Silica gel G is taken in a beaker and made into the slurry with distilled water. The plate is then tipped back to spread the slurry uniformly over the surface. These plates are air dried before being heated to 110 °C for 30 minutes to activate TLC plates. TLC utilizes a variety of solvents to investigate various chemicals found in preparations **(Chatwal et al., 2011) [14].**

1.7 ANTIMICROBIAL ACTIVITY (Shah, G.C *et al* 2006) [12]

1.7.1 Agar well diffusion method

Preparation of agar media

- 250ml of distilled water and 9.5gm of MHA agar suspended in a 500ml conical flask will be added.
- After that, it will be heated on a hot plate while being stirred often until it dissolves entirely.
- After that, the media will undergo hour-long autoclave sterilization at 121°C.

Procedure

Mueller-Hinton agar (MHA) in a volume of around 25 ml will be poured into a sterile Petri plate and let set. Using a sterile spreader, 50 microliters of bacterium inoculums will be applied to the solidified MHA media. Four 5mm diameter wells will be made in the agar in every one of these plates using a sterile cork borer. Then, 100 microliters of each extract would be added separately to wells and left to diffuse at room temp. Working concentrations of 25 mg, 50 mg, 75 mg, and 100 mg dilution will then be prepared from 500 mg/ml of each extract's stock solution. Standard (Gentamicin, amikacin, tetracycline, and ofloxacin) will be used as the positive control, while an equal proportion of alcohol will serve as the negative control. The plates will be incubated for 24 hours at 37°C, and the radius scale will be used to record

and measure the diameter (in mm) of the inhibition zones of growth inhibition. (Natarajan *et al*., 2010**)**

Test organism:

- A Gram-negative bacterium: *E. coli*
- A Gram-negative bacteria: *Pseudomonas*

2.0 Results

2.1 Phytochemical screening of various extract

S. No.	Phytochemical tests	Hydroalcoholic extract
1.	Carbohydrate	-
2.	Fats & oils	+
3.	Protein & amino acid	+
4.	Glycosides	+
5.	Phytosterol	-
6.	Alkaloids	-
7.	Flavonoids	+
8.	Phenols	+
9.	Saponin	+

Note: (+) means positive, (-) means negative

2.1Thin layer chromatography of hydro-alcoholic extract of *Artemisia roxburghiana* is presented in Table 2.1

Table 2.1 TLC of hydro-alcoholic extract

S.NO.	Extract	Solvent System	Rf Value
1	Hydro-alcoholic	n-butanol: acetic acid: water(4:2:6)	0.35,0.75,0.58,1.03
		n-butanol: acetic acid: water(5:1:4)	0.61, 0.72, 0.76

Fig. 2.1 TLC of hydro-alcoholic extract in solvent system n-butanol: acetic acid: water (4:2:6) (5:1:4)

2.3 To evaluate In –Vitro antimicrobial activity on different standard drugs (antibiotics) of *Artemisia roxburghiana* by agar well diffusion method.

Table 2.2 Standard drug (antibiotic) against *E. coil*

S. No.	Antibiotics	Concentration	Zone of inhibition
1.	Tetracycline	100µg/ml	29mm
2.	Amikacin	100µg/ml	28mm
3.	Gentamicin	100µg/ml	28mm

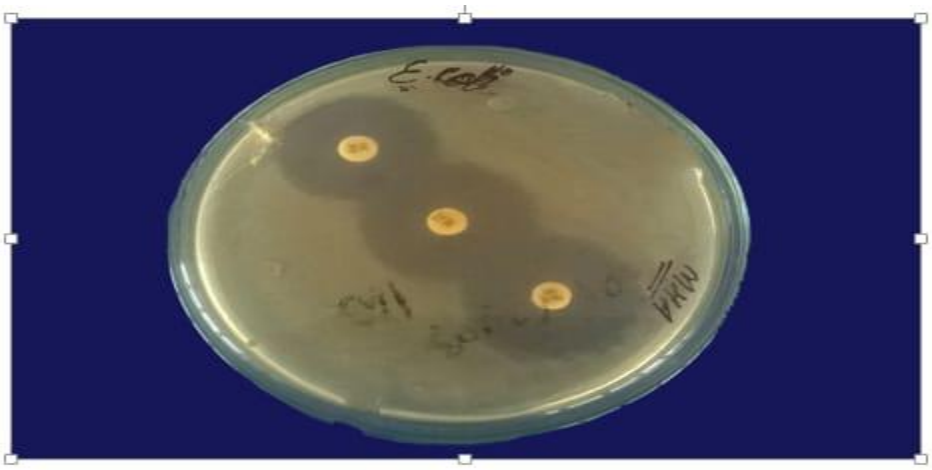

Fig.2.3 Zone of inhibition standard drugs against *E.coli.*

Table 2.3 Standard drug against *Pseudomonas*

S. No.	Antibiotics	Concentration	Zone of inhibition
1.	Tetracycline	100µg/ml	11mm
2.	Ofloxacin	100µg/ml	19mm
3.	Gentamicin	100µg/ml	17mm
4.	Amikacin	100µg/ml	Nil
5.	Amoxicillin	100µg/ml	Nil

Fig.2.4 Zone of inhibition standard drugs against *Pseudomonas*

2.4 Antimicrobial activity on different Extracts of Artemisia roxburghiana by agar well diffusion method.

Table 2.4 Different extracts of *Artemisia roxburghiana* against *Pseudomonas* against *E.coli*

S. No.	Extracts	Concentration	Zone of inhibition
1.	Petroleum ether	100µg/ml	Nil
2.	Chloroform	100µg/ml	Nil
3.	Ethyl acetate	100µg/ml	Nil
4.	Hydroalcoholic	100µg/ml	15mm

Fig.2.5 Zone of inhibition of various extracts of *Artemisia roxburghiana* against *E.coli.*

Table 2.5 Different extracts of *Artemisia roxburghiana* against *Pseudomonas* against *pseudomonas*

S. No.	Extracts	Concentration	Zone of inhibition
1.	Petroleum ether	100µg/ml	Nil
2.	Chloroform	100µg/ml	Nil
3.	Ethyl acetate	100µg/ml	17mm
4.	hydroalcoholic	100µg/ml	Nil

Fig. 2.6 Zone of inhibition different extract of *Artemisia roxburghiana* against *Pseudomonas*

CONCLUSION

The contemporary study has been accomplished with a depiction of the Aerial part of plants. The hydroalcoholic extract is also dynamic as also has phenolics, saponins & flavonoid compounds which are helpful in antimicrobial activity.

Hydro-alcoholic extracts of Aerial parts of plant *Artemisia roxburghiana* showed more effect on reducing microbial growth & oxidation as well as compared to petroleum ether, chloroform & ethyl acetate. However future studies could be undertaken to demonstrate the extracting mechanism of action by which extract expend their antioxidant & antimicrobial activity.

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