Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2019, 11(10):40-47

Research Article

ISSN: 0975-7384
CODEN(USA): JCPRC5

Antimicrobial Activity of *Azadiracta indica*, *Lawsonia inermis* and *Aloe barbadensis* Leaves against Some Multidrug Resistant Microorganisms

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ABSTRACT

Medicinal plants being the valuable source of number of drugs, antibacterial and antifungal activity of Azadiracta indica, Lawsonia inermis and Aloe barbadensis leaves against eight bacterial strains and four fungal strains at three different concentrations 100 µg/ml, 1000 µg/ml and 2000 µg/ml was analysed. Fresh samples of henna, neem and aloe vera leaves 50 gms each were collected and grinded thoroughly with ethanol and soaked for an hour. Fresh juice was further filtered off using Whatmann filter paper No.1, dried using water bath and stored below-20° C. The samples were further investigated for its antimicrobial activity using Kirby-Bauer method. Significant activity observed against all the twelve microbial strains. Henna maintains greater susceptibility against the bacterial strains compared to neem, even at its minimum concentration (100 µg/ml). Against gram -ve multidrug resistant E. coli, henna exhibits antibacterial activity equivalent to that of the control. Compared to that of the control, both henna and neem extracts shows better susceptibility against the gram +ve multidrug resistant Enterococcus species. By regular usage of neem, henna and aloevera our defence system will keep us protected against common bacterial as well as fungal infections.

Keywords: *Azadiracta indica*; Ethanolic extract; Antimicrobial; *Lawsonia inermis*; *Aloe barbadensis*

INTRODUCTION

Our ancient ayurveda needs to be renewed back, covers tremendous miracle herbs which brings back even a lost one. Being treasured with medicinal herbs our mother nature has her hidden solution for every problem. Research work is still lagging in this regard which needs to be replenished. Medicinal plants like neem, henna, keezhanelli, tulsi, adathoda, turmeric, aloe vera [1-7] etc. has been investigated for their wonderful cures for numerous ailments. Neem botanically named as *Azadiracta indica* (neem), being a home of over 135 bioactive compounds could be no wonder coined as “sarva roha nivarani” (a panacea for all illness) [8]. Every part of this Meliaceae member finds its

application against various ailments. Azadirachtin an active promising natural compound extracted from the *Azadirachta indica* tree possess antiviral, antifungal, antibacterial and insecticidal properties which have been known for several years. The other active constituents are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Some of the active principle in leaves is nimbandiol, nimbolide, nimbin, Quercetin, nimbanene, 17-hydroxyazadiradione, β-sitosterol, 6-desacetylnimbinene, ascorbic acid, n-hexacosanol, 7-desacetyl-7benzoylazadiradione, 7-desacetyl-7-benzoylgedunin and nimbiol. Phytochemicals present has been reported to have anthelmintic, anti-inflammatory, antiarthritic, spermicidal, antimalarial, immunomodulant, antiseptic, antifertility, diuretic, antitumour, hypoglycaemic, antiulcerogenic, antiviral, analgesic, anticarcinogenic, hepatoprotective, antioxidant, antihypertensive, antipyretic, antigenotoxic and insecticide [8-13]. Traditionally the twigs are being used for dental cleaning. Beyond its contraceptive nature, reports indicate that a regular intake of neem leaves in small amount fight cancer along with other diseases and act as immuno-stimulant. Neem oil could be applied externally as an antiseptic for urticaria and chronic skin diseases like eczema, scabies, ring worm and maggot infested wounds. It is also used for killing lice, fleas, ticks insecticide and bacterial growth in mouth [10].

The Lythaceae member, *Lawsonia inemis* has long been used by many medical practitioners of traditional herbs. The active principle includes lawsone, lacoumarin, 1,3-dihydroxy-6,7-dimethoxyxanthone, 1-hydroxy-3,6-diacetoxy-7-methoxyxanthone, apigenin-4'-glucoside, apigenin-7-glucoside, luteolin-7-glucoside, luteolin-3'glucoside, stigmasterol, β-sitosterol, 1,2-dihydroxy-4-glucosyloxynaphthalene, lawsaritol. It was found to have properties effective against headache, insomnia, burns, bronchitis, boils, lumbago, abortifacient, dysuria, herpes infection, hemicranias, hysteria, nervous disorders, prurigo ophthalmia, bleeding disorder, syphilitis, sores, scalds, amenorrhoea, scabies, gonorrhoea, liver disorders, vulnerary, venereal diseases, dysentery, calculus, smallpox, diuretic, spermatorrhoea, jaundice, enlargement of the spleen, leprosy, calcalous affections, obstinate skin diseases and spleen disease. Antimicrobial activity of *Lawsonia inermis* (henna) is more evident as it has been traditionally used to decorate nails and toes. It is evident to have anti haemorrhagic, intestinal anti-neoplastic, sedative, analgesic, diuretic, antioxidant, anti-inflammatory, tuberculostatic, antiparasitic, cardio-inhibitory, hypotensive, hypoglycemic, immunostimulant, hepato-protective properties [14-22].

Aloe barbadensis (Aloevera) which belongs to Liliaceae family is a succulent, xerophytic, pea-green color plant. Apart from cosmetic applications, it has been used for treating burns, bruises, skin irritations, mouth diseases, pruritis, indigestion, hair loss, type II diabetes, eye disease, arthritis, tumor, liver complaints, spleen enlargement, vomiting, asthma, jaundice and bronchitis. Besides, it also helps to relieve constipation, maintains a good gastric pH, and helps in inflammatory bowel diseases, non-ulcer dyspepsia, gastric and duodenal ulcers, psoriasis and even leprosy. The plant possess antitumour, anticancer, antiarthritic, antirheumatic, antidiabetic properties and regulates our immune system [23,24]. Some of the active principle responsible for its pharmacological activity includes aloin, emodin, alprogen, C-glucosyl chromone, auxins, gibberellins, aliiase, alkaline phosphatase, amylase, bradykinase, carboxypeptidase, catalase, cellulase, lipase, peroxidase, cholesterol, campesterol, β-sisosterol, lupeol calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc [25].

Traditionally medicinal herbs have been considered as a natural cure for every disease and symptoms. Good old books' regarding natural herbs authored by ancient rishis has been destroyed and also many herbs are categorized as

an extinct one nowadays. On the other hand, complications due to unknown cause are increasing. Synthetic drug usage is reducing the human life span since we are forced to experience the harmful side effects also. Moreover, multidrug resistant bacterial species are increasing nowadays. Research on medicinal herbs is urgency as for the present situation. The present study concentrates on the antimicrobial activity of neem, henna and aloe vera against some bacterial (gram +ve and gram -ve) and fungal species under varied concentrations.

EXPERIMENTAL SECTION

Extraction from Plant Materials

Fresh leaves of neem, henna and aloe vera were collected, grinded well along with ethanol using mortor and pestle. After one hour soaking, the fresh ethanolic extracts were filtered using Whatmann No.1 filter paper. The collected filtrates were dried using water bath at 45°C. The residue was air dried for 30 minutes and further stored below -20°C for testing purpose.

Tested Microorganisms

Antibacterial activity of the ethanolic extracts of *Azadiracta indica, Lawsonia inermis* and *Aloe barbadensis* leaves was tested against eight different bacterial strains *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Shigella sonnei, Enterococcus* species, *Enterobacter* species and *Staphylococcus aureus* at three different concentrations 100 µg/ml, 1000 µg/ml and 2000 µg/ml. Similarly the inhibitory action against four different fungal strains *Candida albicans, Candida tropicalis, Candida cruzi* and *Candida parapsolis* was also analyzed.

Antimicrobial Assay

About 0.01 ml of extract should be poured into Petri dishes on a flat horizontal surface to a depth of 4 mm (25 ml in an 85 mm circular dish, 60 ml in a 135 mm circular dish). The poured plates were stored at 4°C and used within one week of preparation. Before inoculation, plates should be dried with lipids jar so that there were no droplets of moisture on the agar surface. The pH of the medium should be checked at the time of preparation and should be 7.2 to 7.4.

At least four morphologically similar colonies from an agar medium were touched with a wire loop and the growth was transferred to a test tube containing 0.01 ml of sterile suitable broth. The tubes were incubated for 2 hours at 35 to 37°C to produce a bacterial suspension of moderate turbidity. Plates were inoculated within 15 minutes of preparation of the suspension so that the density does not change. After the inoculums have dried, single discs were applied with forceps, a sharp needle or a dispenser and pressed gently to ensure even contact with the medium. When fastidious organisms were to be tested touch multiple colonies with a loop and cross streak the appropriate plate for uniform distribution. It was repeated for each antimicrobial agent to be used, placing the impregnated discs in their respectively labeled segments. After 24 hours, the diameters of the inhibition zones were measured to the nearest millimeter with vernier calipers (preferably) or a thin transparent millimeter scale. For fungal strains same method was followed but the period of time was 48 hours.

RESULTS AND DISCUSSION

The ethanolic extract of all the samples neem, henna and aloe vera leaf exhibit significant antibacterial activity against all the eight bacterial strains *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus*

mirabilis, Shigella sonnei, Enterococcus species, *Enterobacter* species, *Staphylococcus aureus* even at the minimum concentration of 100 µg/ml (Table 1). In particular, at this concentration henna exhibits its optimum activity against all the microbial species except against *Enterococcus* spp. Moreover, its antibacterial activity is found to be superior against gram –ve bacterias *Klebsiella pneumonia, Pseudomonas aerugenosa* and *Enterobacter* species and gram +ve bacteria *Staphylococcus aureus* compared to the other two leaf sample. Against the multidrug resistant gram-ve *E. coli* henna proved to be better, compared to neem and exerts antibacterial activity (19 mm) equivalent to that of the control. Against the gram +ve *Enterococcus* spp. higher activity has been recorded by both neem (13 mm at 100 µg/ml) as well as henna (14 mm at 2000 µg/ml), compared to that of the control (Amikacin). Neem shows greater susceptibility against *Pseudomonas aerugenosa, S. aureus* and *Enterobacter* spp. only at higher concentration (1000 µg/ml). For its optimum activity against *E. coli* and *Enterobacter* spp., still a higher concentration may be required.

Table 1. Antibacterial activity of ethanolic extract from *Azadiracta indica, Lawsonia inermis and Aloe barbadensis*

	Zone of Inhibition Diameter (mm)									
	***Azadiracta indica* leaf**			***Lawsonia inermis* leaf**			***Aloe barbadensis* leaf**			**Control (Amikacin)**
Bacterial strains	100 µg/ml	1000 µg/ml	2000 µg/ml	100 µg/ml	1000 µg/ml	2000 µg/ml	100 µg/ml	1000 µg/ml	2000 µg/ml	
Escherichia coli	12	13	14	19	14	14	8	13	11	19
Klebsiella pneumoniae	9	10	9	16	9	11	7	11	12	23
Shigella sonnei	14	15	14	16	11	14	8	13	14	20
Pseudomonas aeruginosa	9	15	12	14	13	12	8	13	12	20
Enterobacter spp.	6	12	13	13	10	10	7	13	11	21
Proteus mirabilis	13	12	8	12	12	11	8	9	9	21
Enterococcus spp.	13	13	10	10	10	14	8	10	10	12
Staphylococcus aureus	9	12	11	13	14	11	7	7	10	25

Comparing the three leaf samples, highest activity is recorded for henna whereas lowest activity is for aloe vera (Figure 1). Moreover, for aloe vera a higher concentration is required for its optimum activity especially against *S. aureus* (2000 µg/ml).

Against all the four fungal strains all the three leaf samples, exhibits significant activity (Table 2). Compared to aloe vera, both henna and neem shows appreciable suceptibility against all the four strains (Figure 2) even at its minimum concentration (100 µg/ml). Particularly neem records higher activity against *C. albicans* (15 mm at 1000 µg/ml). For aloe vera, better activity is observed against all the four species only at a higher concentration (1000 µg/ml).

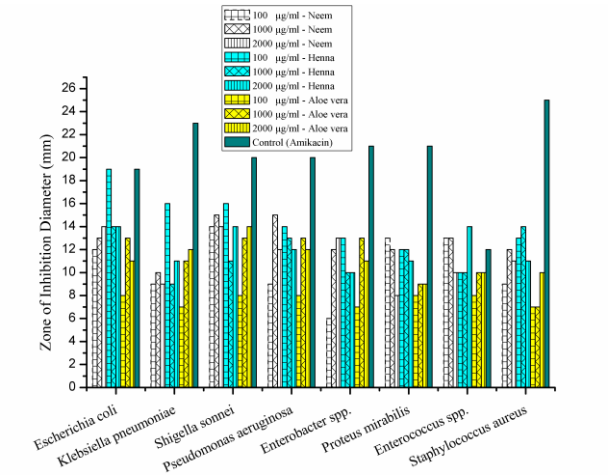

Figure 1. Antibacterial activity of ethanolic extract from *Azadiracta indica, Lawsonia inermis and Aloe barbadensis*

Table 2. Antifungal activity of ethanolic extract from *Azadiracta indica, Lawsonia inermis* and *Aloe barbadensis*

Zone of Inhibition Diameter (mm)										
	***Azadiracta indica* leaf**			***Lawsonia inermis* leaf**			***Aloe barbadensis* leaf**			
Fungal strains	**100 µg/ml**	**1000 µg/ml**	**2000 µg/ml**	**100 µg/ml**	**1000 µg/ml**	**2000 µg/ml**	**100 µg/ml**	**1000 µg/ml**	**2000 µg/ml**	**Control (Flucanazole)**
Candida albicans	12	15	12	11	10	11	9	10	11	29
Candida tropicalis	13	14	11	14	14	12	8	10	11	34
Candida parapsolis	12	12	11	12	13	14	8	11	12	24
Candida cruzi	12	13	13	10	10	10	7	12	10	30

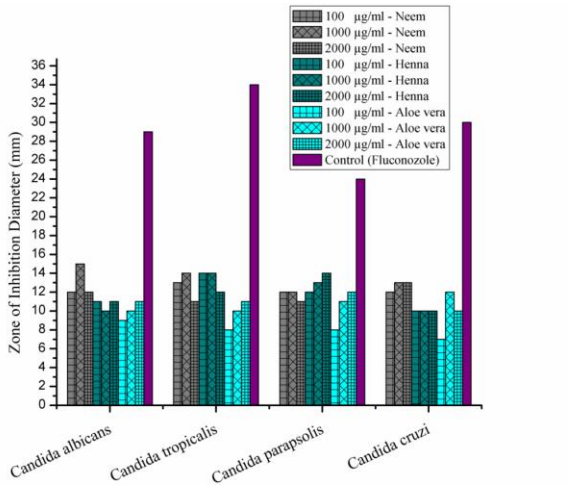

Figure 2. Antifungal activity of ethanolic extract from *Azadiracta indica, Lawsonia inermis and Aloe barbadensis*

Traditionally, neem has been proved to be omnipotent "Divine Tree" because of its chemically diverse and structurally complex secondary metabolites [26]. Secondary metabolites like triterpenoids, flavonoids, polyphenols, tannins etc. has been the cause for the antimicrobial property reported so ever [27,28]. More than 130 bioactive

compounds such as desactylimbin, quercetin, quercitrin, morin, sitosterol, meliantriol, azadiractin, nimbin, nimbidin, nimbinin, nimbosterol, margisine has been reported in neem [9,29-35]. Being a miracle tree among the floral kingdom, reports point out that regular intake of neem leaves prevents the approach of infectious diseases, by stimulating the defence mechanism of our body. But the variation in antibacterial activity reported here may be due to the higher solubility of quinone (lawsone) in henna, meant for its antimicrobial activity as well as its dyeing property. High protein binding capacity of lawsone has been reported to be the reason for its activity [36]. It has been reported that neem leaf contains higher hydrocarbon compounds (85.36%) compared to oxygenated compounds. Free radical scavenging ability has been reported to be lower compared to its flowers which contain 28.3% total oxygenated compounds [37].

Apart from Lawsone the main constituent responsible for the antimicrobial property of henna, number of bioactive compounds have been analysed of which the most important includes mucilage, mannite, gallic acid and tannic acid [16]. Antimicrobial properties of aloe vera has been reported due to the presence of bioactive compounds like anthraquinones which includes emodin, aloetic acid, alovin, anthracine and enzymes like anthranol, barbaloin, chrysophanic acid, smodin, ethereal oil, ester of cinnamonic acid, isobarbaloin and resistannol [38].

Microorganisms have been proved to gain resistance towards modern drugs. Multiple drug resistant bacterias such as gram –ve *E. coli, Klebsiella pneumoniae, Pseudomonas aerugenosa, Enterobacter* spp. etc., and gram +ve bacteria such as *Enterococcus* spp., *Staphylococcus aureus* etc., needs to be controlled by novel bioactive compounds [39,40]. Bacterial infections such as bacteremia (caused by *Staphylococcus aureus*) [41], urinary infection (caused by *E. coli*) [42] are proved to be lethal for infants. Moreover, these infections are reported to be common too. An US record point out that gram-ve bacterial infections are the most predominant among which *E. coli* is the most common bacteria recorded in hospitalized patients followed by *P. aerugenosa, Klebsiella* species, *Enterobacter* species etc. [43].

Among fungal infections, *Candida* species has been reported to be the main culprit targeting mainly our skin. In particular, *C. albicans* has been investigated to be the common type. Unless our immune system functions properly, the particular yeast infects our skin, mouth, intestinal tract, vagina and other mucous membranes which progressively migrate into blood and membranes around heart and brain [44]. Our nature's gift well exerts activity against all the selected fungal species. Our ancestors rightly practiced the usage of henna for decorating our hands and toes to fight against these fungal infections. Fungal infections due to *Candida* species could be better eradicated by using the leaf samples especially neem and henna in our daily life.

CONCLUSION

World Health Organization has reported that medicinal plants would be the best source for exploration of novel drugs which could eliminate the possible side effects which otherwise be a problem of concern while using synthetic drugs. Moreover such innovations will be cheaper and environmentally benign. In the present study apart from exhibiting appreciable antibacterial and antifungal activity against all the selected strains, neem and henna shows pronounced activity against and equivalent to that of the control. Against the multidrug resistant *E. coli*, henna reports equivalent activity to that of the control amikacin. Both the medicinal plants exert better activity against

Enterococcus species compared to that of the control. Neem, henna and aloe vera being a natural cure for bacterial as well as fungal infections, can replace modern drugs for which safety too remains a matter of concern globally.

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