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Antimicrobial Potential of *Ocimum sanctum* and *Adhatoda vasica*: The Medicinal Herbs

Mishra. T* and Rai. A
Assistant Professor* and Research scholar, Department of Botany, Deen Dayal Upadhaya Gorakhpur University, Gorakhpur, Uttar Pradesh, India-273009.

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***Corresponding Author Email:** tulika.mishra.2000@gmail.com

Abstract
In the present review an attempt has been made to congregate the ethnomedicinal, botanical, phytochemical, and toxicological information on *Ocimum sanctum* and *Adhatoda vasica*, the medicinal herbs used in the indigenous system of medicine. The antimicrobial potential of *O. sanctum* and *A. vasica* against some selected human pathogens causing infection have been evaluated by taking different extracts. Agar well diffusion method has been employed for evaluation of antimicrobial potentials. The minimum inhibitory (MIC) concentration of test agents was determined by serial dilution method. Aqueous extract of Tulsi has shown inhibitory effect on *E. coli*, *Bacillus subtilis, Salmonella newport, Proteus vulgaris, Klebsiella aerogens* while ethanolic extracts of *Adhatoda vasica* was found to be more effective. Alcoholic and ethanolic extracts of *O. sanctum* and *A. vasica* shown inhibitory effect against *Vibrio cholerae*. Antifungal effect shown by aqueous and methanolic extract of *O. Sanctum* and *A.vasica* against *Candida albicans, Candida tropicalis* and *Cryptococcus marinus*. Different chemical constituents of Tulsi such as apigenin, linalool and urosolic acid exhibited antiviral effect against Herpes simplex virus (HSV-1 and HSV-2), Adeno virus (ADV-8), Coxsackie virus B1 (CVB), enterovirus 71 (EV 71). Antiviral potential of *A. vasica* also shown by aqueous and methanolic extracts more effectively in Influenza virus. Therefore, it can be concluded that *O. sanctum* and *A. vasica* possess antimicrobial activity which can be further explored for the development of formulation and their structural elucidation.

Keywords
Antibacterial, Antifungal, phytochemicals and Antiviral.

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INTRODUCTION
India is the botanical garden of the world because of the vast geographical area of the medicinal plants. Herbal botanicals have several medicinal important compounds such as flavonoids, alkaloids, essential oils, peptides, phenols, and unsaturated long chain fatty acids, which show antimicrobial activities.[1] These phytochemicals can be used as a drug against the various diseases. The major advantage of plant-based natural drugs over synthetic drug is that natural drugs are safe, have fewer side effects and are biodegradable. According to the World Health Organization, around 80% of the world's population use plants for their primary health care.[2]

The medicinal use of plants is very ancient. The therapeutic use of plants is as old as 4000– 5000 B.C as the writings indicate and the Chinese was the first who initiated the use of the natural herbal preparations as medicines. In India, however, earliest references according to the use of plants as medicine appear in Rigveda which is said to be written between 3500–1600 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians in Ayurveda (an indigenous system of

medicine) which is a basic foundation of ancient medical science in India.[3]

Ocimum sanctum known as Tulsi or Holy Basil is cultivated abundantly for the religious and medicinal purposes throughout the old world and especially in the tropics. Tulsi is also known as "the elixir of life" since it promotes longevity. Different parts of plant are used in Ayurveda and Siddha Systems of Medicine for prevention and cure of many illnesses. Within the Hindu Vaishnavite tradition the worship of devotees involves the use of this plant. Tulsi has been described in Ayurveda as Sashemani Shwasaharani (antiasthmatic) and Kaphaghna (suppressant drug).[4,5.6]

Adhatoda vasica is a highly valued Indian medicinal plant belongs to Acanthaceae family. It is also known as *Justicia adhatoda* (Linn.), Vasaka and Malabar nut. *Adhatoda va*sica is a well-known expectorant in both Ayurvedic and Unani Systems of Medicine.[7] In Ayurvedic preparations, Vasaka leaf juice (Vasa swarasa) is incorporated in more than 20 formulations.[8] It is a primary herb of the ayurvedic system used in the treatment of coughs, bronchitis, asthma and symptoms of common cold. A yogic practice is to chew the leaf buds alone, or with a little ginger root, to clear the respiratory passages in preparation for the vigorous breathing exercises. It is used as an ingredient in numerous popular formulations, including cough syrups, in which it is frequently combined with tulsi (holy basil) and ginger.[9]

Description of plant

Ocimun sanctum

Ocimum sanctum belongs to Family Labiaceae. It is found throughout tropical and semitropical region of India and other Asian countries. [10,11] It is a branched, fragrant and erect herb and a mature plant attains height of about 75 to 90 cm, **Fig. 1**. Its leaves are nearly round and up to 5 cm long with margin, i.e. entire or toothed. Flowers are small having purple to reddish colour, present in small compact cluster or cylindrical spike. The arrangement of flower is verticillaster and counted in unique morphological properties of this plant, **Fig. 2**. The fruits are small and yellow to reddish in colour.

Scientific classification [11] Kingdom: Plantae
(unranked): Angiosperms
(unranked): Eudicots
(unranked): Asterids
Order: Lamiales
Family: Lamiaceae
Genus: *Ocimum*
Species*: O. sanctum*

Figure 1: *Ocimum sanctum*

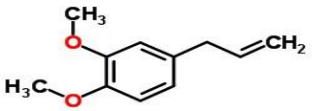
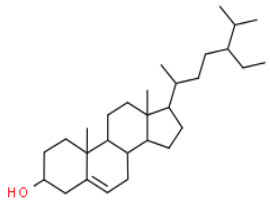
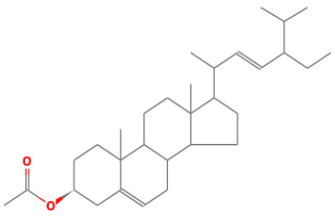
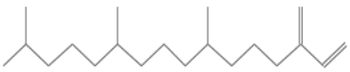
Figure 2: Verticillaster inflorescence

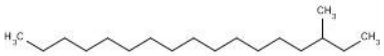
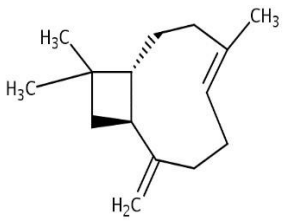
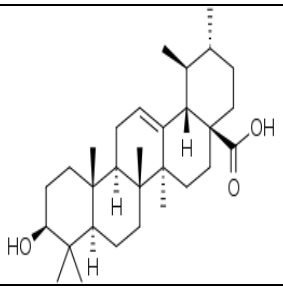
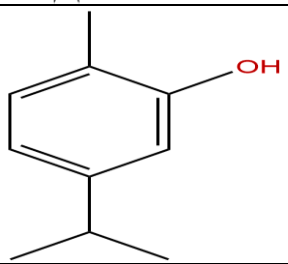
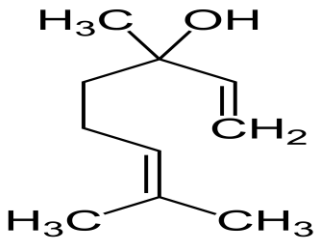
Phytochemical constituents

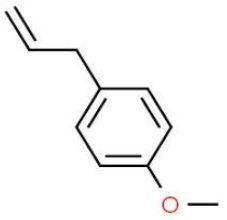
The chemical composition of *Ocimum sanctum* (Tulsi) is a complex of many nutrients and some commonly recognized biologically active compounds those are involved in pharmacological activities against different disease conditions.[12] Different preparations (dried leaf powder, methanolic, acetonic and petroleum ether extracts) are obtained from leaves

of *O. sanctum* on the basis of Gas chromatography and mass spectrophotometery (GC-MS). In dried leaf powder 49 components were found, major components were 1-Methyl eugenol (89.20%),2-Eugenol (5.29%), in methanolic extract 1-Stigmast-5-en-3-ol (17.46%), 2Stigmast-5, 22-dien-3-ol (13.13%), 3-Methyl eugenol (6.19%) were found in majority, in acetonic extract 1- Methyl eugenol (25.31%) and 2-Neophytadiene (7.77%) were found in majority, in petroleum ether extract 1- Methyl eugenol (20.97%), 2-Octadecane (17.50%), 3β-caryophylene (8.22%) were found in majority.[13] The leaf volatile oil contains eugenol (1hydroxy-2-methoxy-4-allylbenzene), euginal (also called eugenic acid), urosolic acid, carvacrol (5-isopropyl-2-methylphenol), linalool (3,7-dimethylocta-1,6-dien-3-ol), limatrol, caryophyllene, methyl carvicol (also called Estragol: 1-allyl-4-methoxybenzene) while the seed volatile oil have fatty acids and sitosterol. Other than these the seed mucilage of Tulsi contains some levels of sugars and green leaves are the source of anthocyans. Two major sugars of the plants are xylose and polysaccharides.[14,15] The aqueous extract of *O. sanctum* leaves revealed alkaloids, flavonoids, tannins and carbohydrates.[16,17]

Table 1: Structure of phytochemicals of *Ocimum sanctum*

S.NO	Name of compound	Structure
1.	1-Methyl eugenol	
2.	1-Stigmast-5-en-3-ol	
3.	2-Stigmast-5, 22-dien-3-ol	
4.	2-Neophytadiene	

5.	Octadecane	
6.	3-β-caryophylene	
7.	urosolic acid	
8.	Carvacrol	
9.	Linalool	

10.	Estragol	

Antibacterial Properties

Basically, in vitro studies are performed against specific pathogens under laboratory conditions and theses are correlated with the growth or the inhibition of the pathogen. In majority of cases dose dependent effects are observed for a specific period of time.[18,19] In this series essential oil from the leaves exhibited some inhibitory effect against *E. coli, Bacillus anthracis, B. subtilis, Salmonella newport, Sal. pullorum*, *Staphylococcus aureus, Proteus vulgaris and P. aeruginosa.*[20] These essential oils include major constituents of leaves such as eugenol, methyl eugenol and caryophyllene. Among them all except caryophyllene were reported to inhibit the growth of *Arthobacter globiformis*, *Bacillus megatherium, E. coli and Pseudomonas sp.* Eugenol was found most effective constituent.[21,22] The antibacterial effect of aqueous and alcoholic extracts of O. sanctum leaves are supposed to be more efficient as these are recommended in most of traditional medicinal systems. Similarly, these were found effective against various enteric pathogens viz., *E. coli, Klebsiella aerogens, Proteus mirabilis, Salmonella typhimurium, Shigella dysentriae, Pseudomonas aeruginosa, Vibrio cholera and Staphylococcus aureus.*[23] The aqueous extract showed wider zones of inhibition for *Klebisella* spp., *E. coil, Proteus spp.* and *Staphylococcus aureus* whereas alcoholic extract showed wider zone for *Vibrio cholera.*[24] Other than leaves the seeds of Tulsi were also exploited for antibacterial activities and crude, supernatant, residue and dialyzed samples obtained from the seeds inhibited the growth of *P. multocida, E. coli, B. subtilis* and *Staph. aureus*. The minimum inhibitory concentration (MIC) values of extracts revealed that *Pasturella multocida* and *Bacillus subtilis* were most sensitive strains.[25] The aqueous extracts were found more active than methanolic extracts.[26,27,28,29]

Antifungal properties

Fungal pathogens are always hard nuts to crack in medical sciences. The pathogens involved in human and animal ailments are more or less present all over world and are posing a severe threat due to drug resistance and through reoccurrence of diseases. It is commonly observed that these disease conditions are more or less remains life long without cure. Even the use of allopathic medicines provides temporary relief with a threat of side effects to patients.[28] Thus, the use and effectiveness of antifungal activity of medicinal plants is lightening a hope against antifungal diseases. Candidiasis is a very common disease not only in human but also in animals and therefore always has been a challenge to scientist. Ethanolic extracts of *O. sanctum* (whole plant) were reported to have 21-30mm zone of inhibition against *Candida albicans* [30] and were less effective in comparison to aqueous extract.[24] In contrast to these there are also some reports with almost negligible effects of chloroform, acetone and methanolic extracts of

O. basilicum L. against *Candida albicans* ATCC 845981, *Candida crusei* ATCC 6258, *Candida albicans* ATCC 90028.[31] Leaves extracts have been found effective in controlling *Fusarium solani* f. sp. Melongenae.[32] Similarly, these were also found to inhibit the *A. flavus* growth (65–78%) and AFB1 production (72.2–85.7%).[33]

Antiviral properties

Viruses are obligate intracellular parasites and use host cell machinery for their survival, thus any drug or drug delivery system involved in control and treatments of viral diseases might interact with cells and cellular mechanism and thus may produce adverse effect. These mechanisms and cellular functions are vital for the survival of host cell system and thus there are always needs of nontoxic antiviral agents which do not have adverse effects on the normal cellular mechanism. Irrespective of the type of nucleic acid, viruses are sensitive to different constituents of Tulsi extracts as purified components, namely apigenin, linalool and ursolic acid, which exhibited a broad spectrum of antiviral activity against DNA viruses viz. Herpes viruses (HSV), Adenoviruses (ADV), Hepatitis B virus and RNA viruses viz. Coxsackie virus B1 (CVB1), Enterovirus 71 (EV71); the strongest activity was recorded with ursolic acid against HSV-1 (EC50 = 6.6 mg/L; (SI) = 15.2), ADV-8 (EC50 = 4.2 mg/L; SI = 23.8), CVB1 (EC50 = 0.4 mg/L; SI = 251.3) and EV71 (EC50 = 0.5 mg/L; SI = 201), whereas apigenin showed the highest activity against HSV-2 (EC50 = 9.7 mg/L; SI = 6.2), ADV-3 (EC50 = 11.1 mg/L; SI = 5.4), hepatitis B surface

antigen (EC50 = 7.1 mg/L; SI = 2.3) and hepatitis B antigen (EC50 = 12.8 mg/L; SI = 1.3) and linalool showed strongest activity against AVD-II (EC50 = 16.9 mg/L; SI = 10.5) [34], where EC50 is the half maximal effective concentration of drug and SI is the selectivity index.

Table 2: Antimicrobial activity of chemical extracts of *Ocimum sanctum*

Potential	Chemical Constituents	Pathogen
Antibacterial	Eugenol>methyl-eugenol>caryophyllene	*A. globiformis, B. megatherium, E. coli, Pseudomonas spp.*
	Aqueous extract of leaves	*Klebisella spp, E. coli, Proteus spp, Staphylococcus aureus*
	Alcohlic extract of leaves	*Vibrio cholera*
Antifungal	Ethanolic extract	*Candida albicans*
	Leaves extract	*Fusarium solani F.sp.*
Antiviral	Urosolic acid	HSV-1, ADV-8, CVB-1, EV7
	Apigenin	HSV-2, ADV-3, Hepatitis B surface antigen
	Linalool	ADV II

Adhatoda vasica

It is a common small evergreen, sub-herbaceous bush distributed throughout India, especially in the lower Himalayas (up to 1300 meters above sea level), India, Sri Lanka, Burma and Malaysia. *Adhatoda* leaves have been used extensively in Ayurvedic Medicine for over 2000 years primarily for respiratory disorders. Charaka Samhita has classified the drug under mucolytic and expectorant drug. The roots, leaves & flowers are active principles of the plant possess a number of pharmacological properties & are used in cough, chronic bronchitis, rheumatism, asthma & bronchial asthma.[35] It is a dense shrub 1.2-2.4 m **Fig. 3**. sometime arborescent, 6m. High with many long opposite ascending branches; stem with yellowish bark, terete, glabrous. Leaves are elliptic-lanceolate, acuminate, minutely puberulous when young, glabrous when mature, entire, dark green above, paler beneath, base tapering; main nerves 1012 pairs with reticulate venation between; petioles 1-2.5 cm long. Flowers in short dense axillary pedunculate spikes long **Fig. 4**, towards the end of the branches; peduncles , stout, shorter than the leaves; bracts reaching 1-2 by 0.5-1.2 cm, elliptic subacute, glabrous or nearly so, 5-7 nerved, closely reticulately veined; bracteoles 1.5-2 by 0.3-0.4 mm., oblonglanceolate, acute, with ciliolate margins, 1-nerved, reticulately veined. Seeds 5-6 mm. long, orbicularoblong, glabrous.[36]

Scientific classification:

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperm
Clade: Eudicots
Clade: Asterids
Order: Lamiales
Family; Acanthacea
Genus: *Adhatoda*
Species: *A.vasica*

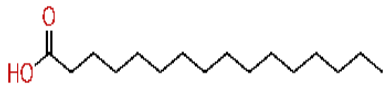
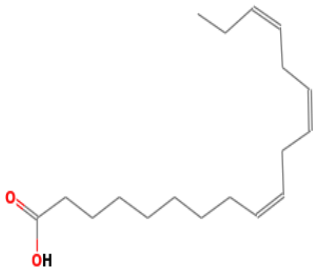
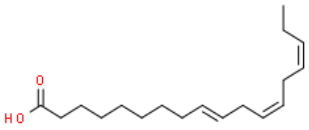
Figure3: *Adhatoda vasica* plant

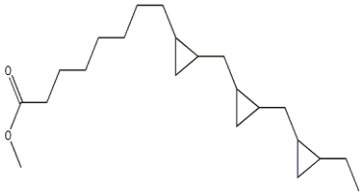
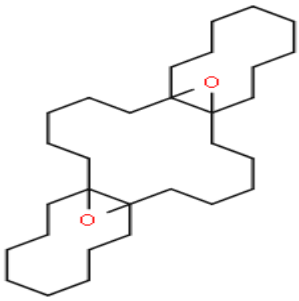
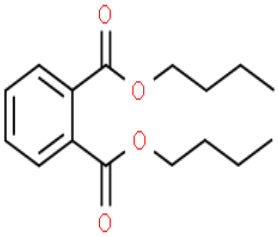
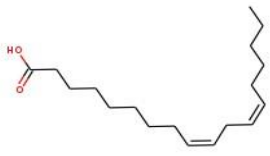
Figure4. Axillary pedunculate spik Flower of *A.vasica*

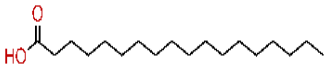
Phytochemical constituents

Identification of phytocompounds was based on the principles of retention time, molecular formula, molecular weight (Mw), and concentration. It is done to determine some compounds present in plants having any medicinal value. The Gas chromatography and mass spectrum analysis of leaf, shoot, and flower extract revealed the existence of several compounds. The major compounds identified in *A. vasica* in terms of area percentage(%) are 9,12,15octadecatrienoic acid, methyl ester (26.76), 9,12,15-octadecatrienoic acid, linolenic acid (19.44), hexadecanoic acid, methyl ester (16.82), cyclopropaneoctanoic acid, methyl ester (9.69), methyl eicosatetraenoate(6.03), and binaphthyl sulfone (2.16), mannitol, 1,3,4,5-tetraO-methyl diacetate (4.64), methanone, 4-dimethylamino phenyl (3.64) and 1-dimethyl(3chloropropyl) silyloxyoctane (1.46). The most studied chemical component is a bitter quinazoline alkaloid, vasicine, which is present in the leaves, roots, and flowers.[37,38,39] Apart from vasicine, important chemical constituents identified in the leaf are vasicol, adhatonine, vasicinone, vasicinol, and vasicinolone.[40,41] The other alkaloids discovered in the plant are adhavasicinone, anisotine, and peganine, betaine, steroids, and alkanes.[37,38,39] It also contains vitamin C, saponins, flavonoids as well as steroids and fatty acids. Essential oils of the leaves of *A.vasica* are also known to contain ketone, terpene, and phenolic ether.

Table 3: Structure of phytochemicals of *Adhatoda vasica*

S.NO	Name of compound	Structure
1.	Hexadecanoic acid	
2.	9,12,15-octadecatrienoic acid, (Z, Z, Z)	
3.	9,12,15-octadecatrienoic acid	

4.	Cyclopropaneoctanoic acid	
5.	9,12-octadecadienoic acid	
6.	Tricyclo 7,16 triacontane	
7.	Dibutyl phthalate	
8.	9,12-octadecadienoic acid (Z, Z)-	

9.	Octadecanoic acid	

Antibacterial properties

The results of the agar-well diffusion method show that the crude ethanolic extract of the leaf exhibits an antimicrobial activity against the test organisms; *S. aureus, S. epidermidis, B. subtilis, and P. vulgaris*, with a maximum diameter of the zone of inhibition ranging from
19 mm, 18 mm, 14 mm and 15 mm. Similarly, the antimicrobial activity of petroleum ether extract against *S. aureusis* observed with a 16 mm zone of inhibition. Other test organisms are highly resistant to petroleum ether and aqueous extracts.[9] This justifies the traditional use of ethanol in extracting the leaf components, to control the pathogenic organisms.[42] The MIC of the ethanolic extract of *Adhatoda vasica* against bacterial pathogens, such as, *S. aureus, S. epidermidis, B. subtilis and P. vulgarisis* observed at 75 mg/ml. Sujogya *et.al* [43] examined that ethanolic extract also exhibited antibacterial activity against *E.coli, S. flexn and V. chloera* with a 10 mm maximum diameter of the zone of inhibition. The MIC of ethanolic extract of *Adhatoda vasica* against *E. coli, S. flexn, V. chloera* observed at 500,1000,2000mg\ml. Josephin and Selva [44] stated that in case of A. vasica, solvents showed higher antibacterial activity in the order of diethyl ether > methanol > ethanol > acetone > Chloroform> water for different clinical pathogens in the order of *Klebsiella pneumoniae>Staphylococcus aureus > Proteus valgaris > Pseudomonas aeroginosa > Streptococcus spp.*

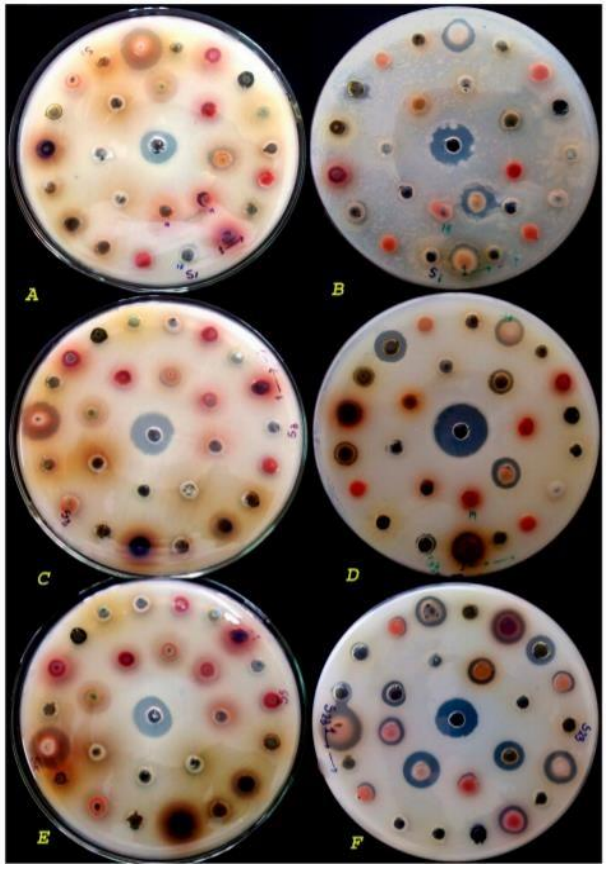


Figure 5: Screening of plant extracts; **(A)** Plant extracts (methanol) against E. coli; **(B)** Plant extracts (water) against E. coli; **(C)** Plant extracts (methanol) against S. aureus; **(D)** Plant extracts (water) against S. aureus; **(E)** Plant extracts (methanol) against S. typhimurium; **(F)** Plant extracts (methanol) against V. cholera.[43]

Antifungal Properties

Screening of invitro antimicrobial activity of methanol, n-hexane and aqueous extracts of *Adhatoda vasica* leaves was done by using selected fungal strain belonging important pathogenic infection like *Candida tropicalis* 3017 and *Cryptococcus marinus* 1029. All extracts of *A.vasica* showed optimum activity against all tested microorganisms. However, results of antifungal activity revealed that methanolic extract showed highest activity followed by n-hexane and aqueous extracts. Methanolic extract showed highest inhibition (20 mm; MIC:78.12ug\ml) and lowest (15 mm; MIC:625ug\ml) against *C.tropicalis* and *Cryptococcus* respectively. Karthikeyan et al.,[9] determined that crude ethanolic extract of the leaf also exhibited antifungal activity against the test organism *Candida albicans* with maximum diameter of the zone of inhibition is 12 mm, with MIC of the ethanolic extract observed at 100 mg\ml. *C.albicans* is highly resistant to petroleum ether and aqueous extract.

Antiviral Properties

The influenza viruses are major etiologic agents of human respiratory infections and inflict sizable health and economic burden. The present study reports the in vitro antiviral effect of *Adhatoda vasica* crude extracts against influenza virus by Hemagglutination (HA) reduction in two different layouts of simultaneous and post treatment assay. The aqueous and methanolic extracts were used for antiviral activity in the noncytotoxic range. Methanolic extract showed 100% reduction in HA in the simultaneous and post treatment assays at the concentration of
10mg/ml. The aqueous extracts at concentrations of 10mg/ml and 5mg/ml reduced the HA to 33% and 16.67%, respectively, in the simultaneous assay. These results suggest that extracts have strong anti-influenza virus activity that can inhibit viral attachment and/or viral replication and may be used as viral prophylaxis.[45]

Herpesviruses are important human pathogens that can cause mild to severe lifelong infections with high morbidity. The aqueous and methanol extract from leaves of *A.vasica*, were used to study the cytotoxicity effect on Vero cell line by using MTT assay. The methanolic extract at 10mg/ml significantly inhibited formation of plaques in Vero cells infected with 100 pfu of HSV1 and 2 by 100%. Similarly, the aqueous extract at 10mg/ml inhibited the plaque formation by 100% and 86% for HSV1 and 2. These results suggest that this herbal extract has potent anti-viral agents against Herpes simplex viruses that can be exploited for development of an alternative remedy for HSV infections.

Table 4: Antimicrobial activity of chemical extracts of *Adhatoda vasica*

Potential	Chemical constituents	Pathogen
Antibacterial	Diethyl ether	Klebsiella pneumoniae
	Ethanolic extract	S. aureus >S.epidermidis> B. subtilis>P.vulgare
Antifungal	Methanol>n-hexane>aqueous extract	Candida tropicalis3017 and Cryptococcus marinus
	Ethanolic extract	Candida albicans
Antiviral	Methanolic extract>aqueous extract	HSV-1 and HSV-2

CONCLUSION

The literature survey revealed that *Ocimum sanctum* and *Adhatoda vasica* both has been widely studying for its phytochemical and pharmacological activities. They present in class of herbal drug with very strong conceptual or traditional base. The present study clearly indicates that *O. sanctum* and *A. vasica* are rich source of phyto-chemical constituents. The antimicrobial efficacy of *O. sanctum and A. vasica* indicates that the plants have potent antimicrobial properties as well as both are widespread in India. They can be recommended as easily available and renewal source of antimicrobial agent instead of synthetic chemicals. Infectious diseases play a significant role in the death of millions of people worldwide, majorly due to the mutagenic nature of the genome of the microbes. Accordingly, it is desirable and essential to develop an effective, safe and natural product to control multiple drug resistance (MDR) pathogen. Further work is required on conservation of these plant species to make them constant in our lives and to also to identify the active bio-compounds. The synergistic effect of their important medicinal properties, toxicity level and activity of the secondary metabolites needs clinical evaluations.

Conflict of interest

There is no conflict of interest in the preparation of this article.

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