Pharmacognostic Evaluation and Antimicrobial Activity of Some Medicinal Plants Extracts Commonly Used in Indian Traditional Medicine

[*1]Mirza Azim Beg and [2] Ragib Ali
[1 & 2] QA/QC Department, Himalaya Wellness Company, Faridabad, Haryana, India
[*]**Email – azim0088@yahoo.com**
DOI 10.51129/ujpah-2022-32-1(9)

Received –May 30, 2022
Revised – June 06, 2022
Accepted – June10, 2022
Published – June 18, 2022

Abstract – Indian traditional medicines have been used to boost health since the time of immemorial and the achievement of contemporary medical science mainly depends on drugs initially obtained from natural resources. In the past, a large number of antimicrobial compounds were discovered from synthetic and natural products for the treatment and control of infectious agents. *Adhatodavasica*, *Tinosporacordifolia, Glycyrrhizaglabra*, *Boerhaviadiffusa, and Eclipta Alba* are ethnomedicinal plant. They are used in different diseases like breathing disorders, burning sensation, Cough, decrease in bone tissue, blood disorders, tuberculosis, as refrigerant, aphrodisiac, in insect bites, rheumatism, as tonic and in general debility. They are vital component of many Ayurvedic formulations. Despite the common utilization of these plants, convincing study required for reporting the pharmacognostic evaluation along with their antimicrobial activity.

Key words: *Adhatodavasica*, *Tinosporacordifolia, Glycyrrhizaglabra*, *Boerhaviadiffusa, and Eclipta Alba*, Pharmacognostic Evaluation, Physico-chemical,Histochemical,Zone of Inhibition, Antimicrobial.

Introduction

A large percentage of the world's population depends upon natural products for medicine. Folk medicine and ecological awareness suggest that natural products are harmless[1]. Therefore trend is shifting from synthetic to herbal medicine, which has been called as 'Return to Nature'[2]. India, have a pluralistic healthcare system. Herbal drugs constitute a major share of all the formally recognized systems of health in India viz. Ayurveda, Yoga, Unani, Siddha,Homeopathy and Naturopathy, except Allopathy. Almost, 70% modern medicines in India are derived from natural products[3]. Natural products sustained to play a highly substantial role in the drug discovery and development process[4]. Medicinal plants play a crucial role not only as traditional medicines but also as trade commodities[5]. The role of information derived ethnomedicine and its utility for drug discovery purposes is important[6]. A lot of work has been done on ethnomedicinal plants in India but still some important plants are still to be scrutinized. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have pharmacological properties[7].

Adhatodavasica:*Adhatodavasica*also known as malabar nut tree is part of the Acanthaceae plant family. It is a small evergreen, sub herbaceous bush which grows commonly in open plains, especially in the lower Himalayas (up to 1300 meters above sea level), India, Sri Lanka, Burma and Malaysia[8]. It is a highly reputed plant used in Ayurvedic system of

medicine for the treatment of various ailments of respiratory systems like bronchitis, asthma and it is also used in the treatment of malaria, dysentery and diarrhea[10] and has many other medicinal applications[9-11], *Adhatodavasica*Linn. Also has anti-inflammatory, analgesic, diarrhoea, dysentery, antioxidant,hepatprotective, Sedative, antispasmodic, anthelmintic properties[12], Antimicrobial activity[13], Antidiabetic activity[14], Wound healing effect[15]. Infertility[16]. Antiulcer[17]. Antibacterial[18]. Antihistaminic effect, mode rate hypotensive activity, thrombo- poietic activity[19].

Tinosporacordifolia:*Tinosporacordi-folia*(Wild) Miers, (*Guduchi*) is oneoftheimportantdioeciousplantsbelongstothefamilyMenispermaceae.In Ayurveda, it is designated as*Rasayana*drugrecommendedtoenhancegeneralbody resistance, promote longevity and as antistressand adaptogen[20-21]. This significant plant is also mentioned inimportant Pharmacopoeias[22-23].Phytochemistryof*T. cordifolia*belongs to differentclassessuchasalkaloids,diterpenoid lactones,glycosides,steroids,sesquiterpenoid,phenolics,aliphaticcompoundsandpolysacc- harides[22-24].Threemajor groups of compounds; protoberberine alkaloids,terpenoids, and polysaccharides are consideredas putative active constituentsofthisplant[25-26].*T.cordifolia*iswidelyusedinfolkloricveterinary medicineandtraditionalAyurvedicmedicine in India for its, anti-inflammatory, immune modulatory, antipyreticactivity,antioxidant,anti-diabetic,antiallergicandantiarthriticactivitiesandvariousother medicinal properties[27-31].

Glycyrrhizaglabra:In*Ayurveda Yashti-madhu*is one of the important plant which is been referred in various texts with many therapeutically uses. *Glycyrrhizaglabra*Linn. a perennial herb with a thick rootstock passing below into long, straight, cylindrical, slightly tapering, smooth, flexible, slightly branched roots, about 1.25cm in diameter, red or orange-brown on the surface, pale yellow within, and giving off at the top long horizontal subterranean stolons. Stems several from the crown, 2-4 feet or more high, erect, stiff, solid, strongly striates, shortly pubescent, branched. Leaves alternate, spreading, large, stalked, with very minute deciduous stipules, impair-pinnate, leaflets opposite in 4-7 pairs and a terminal one[32-33].

***Boerhaviadiffusa*:**Punarnava*(Boerhaviadiffusa*Linn.) is a flowering plant that is commonly known as punarnava which means rejuvenating or renewing the body. Punarnava (Hogweed) literally means 'bring back to life' or 'renewer'. Among 40 species of Boerhaavia, 6 species are found in India, it is a perennial, spreading hogweed, commonly occurring abundantly in waste places, ditches and marshy places during rains. The plant is also cultivated to some extent in West Bengal[34-35]. It grows well on wastelands and in fields after the rainy season[36]. The whole plant and preferably the roots are effectively used to cure several diseases including Jaundice[37]. Punarnava corrects the digestive system, alleviates fluid retention and very useful in managing heart diseases. It is also used to treat the anemia, hernia and respiratory distress, liver problems, managing lipids and cholesterol in healthy limits[38].

Eclipta Alba:*Eclipta Alba*is commonly known as Bhringaraja or Maka belonging to the family Asteraceae/Compositae. The herb contain wedelolactone and demethyl-wedelolactone which possessing potent antihepatotoxic property[39]. Otherprominentchemical constituents present are Ecliptal, Ecliptine, Ecliptalbine, α-Terthienylmethanol, β-amyrin,Sigmasterol, Polypeptides etc. The other pharmacological activities shown by plants are Antiviral, Antibactarial, Spasmogenic, Hypotensive, Analgesic, Antioxidant etc[40].

In the current investigation carried out, pharmacognostic evaluation and screening of different extracts of *Adhatodavasica*,

Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and Eclipta Alba have been used against E. coli and S. Aureus in order to screen new sources of antimicrobial agents.

Material and Methods

Collection and Authentication of Plant Material: *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and Eclipta Alba* were collected from the medicinal plant store of Himalaya Wellness Company Faridabad, Haryana, India, were air dried, powdered and stored in air tight containers. Above these plants were authenticated and identified by Dr. Maya Ram Uniyal Senior vadhya of Himalaya Wellness Company Faridabad. Pharmacognosticevaluations were done as per WHO Guidelines[41].

Chemicals: All reagents and chemicalsused for pharmacognosticevaluation and antimicrobialactivitywere of analytical grade.

PharmacognosticEvalaution: The organoleptic studies were carried out by with sense organs using simple technique like shape, size, colour, odour, taste etc. Histochemical reactions were applied with concentrated hydrochloric acid and phloro-glucinol for identification of lignified elements, iodine solution for starch grains, Sudan red-III for cuticle layer and oil globules, Ruthenium red for mucilage and acetic acid for calcium oxalate crystals.Physicochemical parameters such as loss on drying, ash values, pH value in 1% solution, aqueous, and alcoholic extractive values were carried out according to the methods recommended by the World Health Organization.[19]

Preparation of Plant Extract: After collection of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and Eclipta Alba* samples, they were powdered. Powder materials were passed through sieve no. 40 and used for extractions. Weighed powder was extracted using hexane, chloroform, ethyl acetate, ethanol and aqueous solution in Soxhlet apparatus till exhausted. These extracts was evaporated at 40°C in rotary vacuum evaporator to dryness[20]The extracts obtained from successive extraction *i.e*. Hexane extract (HE), Chloroform extract (CE), Ethyl acetate extract (EAE), Ethanol

extract (EE) and residual Aqueous extract (AAE).

Test Micro-organisms and growth Media: The antibacterial activity of different extracts were studied against two bacterial strains, one Gram-positive (*Staphylococcus aureus* ATTC-6538,) and one Gram-negative (*Escherichia coli*ATCC8739,) based on their pharma-cological importance. Both the strains of micro-organism were obtained from Department of microbiology, Himalaya Wellness company Faridabad, Haryana. The strains of *Staphylococcus aureus* and *Escherichia coli* were maintained on nutrient broth at 37°C and suspension were stored in refrigerator till used**.** Commercially available Mueller-Hinton agar (MHA) (Hi-media, Mumbai) was prepared according to the instructions on the leaflet. Immediately after autoclaving the media, it was allowed to cool. Freshly prepared and cooled medium was poured into glass flat-bottomed petri-plates on a level, flat surface to give a uniform depth of approximately 4 mm. This corre-sponded to 30 ml for each plate with a diameter of 90 mm. The agar medium was allowed to cool at room temperature and unless the plates were used the same day otherwise these were stored in a refrigerator (2 to 8°C) for further use within seven days. Representative samples of each batch of plates were examined for sterility by incubating at 37°C for 24 hours or longer.[42]

Agar Well Diffusion Method for Determination of Zone of Inhibition (ZOI): Antibacterial activity was carried out using well diffusion method. The test cultures were spread with the help of spreader on the top of the solidified media

and allowed to dry. The tests were conducted with 100mg/ml concentrations of these crude extracts per well with three replicates. Dimethyl Sulphoxide (DMSO) (Himedia Mumbai) was used as negative control. Streptomycin discs (10**µg**/disc) of 6 mm were used as positive control. The plates were incubated for 24 h at 37 ºC. Zone of inhibition (ZOI) was recorded in millimetres and the experiment was repeated thrice.The inoculums were prepared by making a direct broth suspension of 24-hour agar plate. The suspension adjusted to match the 0.5 McFarland turbidity standards. Dried extracts were accurately weighed and dissolved in the DMSO to yield the 100mg/ml concentration, using sterile glassware. These were stored in refrigerator for further use. The wells were made in the incubated MHA media plates with the help of sterile cork borer (steel) of 6 mm and plates were labelled properly. 50µl of the working solution of plant extract were loaded into the respective wells with the help of micropipette. The plates were incubated 24 h at 37C. The plates were then observed for the zone of inhibition (ZOI) produced by the anti-bacterial activity of different plant extracts. At the same time ZOI of both organism by different extracts were measured with the help of the ruler for the estimation of effectiveness of anti-bacterial substance and tabulated.

The plates were then incubated in the inverted position at 37°C for 24 h The diameters of the zones of complete inhibition as observed by the unaided eye are measured, including the diameter of the disc/well. Zones were measured to the nearest whole millimeter, using a ruler; these petri plates is held in non-reflecting background and illuminated with reflected light. The zone margin were taken area showing no obvious, visible growth which can be detected with the unaided eye. The same procedure was followed for each strain and extract[43].

Results

Pharmacognostic evaluation:

Pharmacognostic evaluation has been done with respect to Organoliptic properties, histochemical evaluation andphysico-chemical studies.Organoliptic evaluation which is done by sense organs is the simplest and quickest means to ascertain the identity and purity of a drug. Organoliptic characters as shape, size, colour, odour, taste etc. are evaluated. These features of *dhatodavasica*, *Tinosporacordifolia*, *Glycyrrhizaglabra*, *Boerhaviadiffusa*, *and Eclipta Alba* powder samples were observed. The details of results are presented in Table-1.

Table – 1 organoleptic evaluation of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and Eclipta Alba*

SNo.	Plant Name ▶ / Parameter ▼	Observation				
		Adhatodavasica	***Tinosporacordifolia***	***Glycyrrhizaglabra***	***Boerhaviadiffusa***	***Eclipta alba***
1	**Colour**	Greenish brown	Brown	Yellowish	Brown	Brown
2	**Taste**	Bitter	Bitter	Sweet	Bitter	Characteristic
3	**Shape**	Branched herbs	Cylindrical	Cylindrical	Cylindrical	Branched herbs
4	**Odour**	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
5	**Size**	2-6 cm long pieces	2-8 cm long and 1-3 cm diameter	3-9 cm long and 2-4 cm diameter	2-8 cm long and 2-5 cm diameter	2-6 cm long pieces

Histochemical Characters

Powders study by using particular chemicals has been done. The results are presented in Table-2.

Table – 2 histochemical study of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa and Eclipta Alba* powder

S.No.	Reagent	Test	Nature of colour change	Observation				
				Adhatodavasica	***Tinosporacordifolia***	***Glycyrrhizaglabra***	***Boerhaviadiffusa***	***Eclipta alba***
1	Ruthenium red	Mucilage	Pink	-ev	+ev	+ev	+ev	-ev
2	Weak Iodine solution	starch	Blue	-ev	-ev	+ev	-ev	-ev
3	Sudan III	Fixed oil & fat	pink	+ev	+ev	+ev	+ev	+ev
4	Sulphuric acid (60%)	Calcium oxlate	Soluble, on standing show needles of calcium sulphate	+ev	-ev	-ev	-ev	+ev
5	Phloroglucinol-HCl	Lignins	Reddish brown to red rose	-ev	+ev	+ev	+ev	-ev
6	Millon's reagent	Protein	Yellow to brown	-ev	-ev	-ev	-ev	-ev

Physicochemical Analysis

The parameters which have been studied are moisture content, loss on drying, total ash, acidinsoluble ash, alcohol and water-soluble extractive values, foreign matter, and pH analysis. Ash values are useful to indicate presence of various impurities like carbonate, oxalate and silicate. The water soluble ash indicates amount of inorganic compound present in drugs whereas the acid insoluble ash indicate contamination with earthy material. Moisture content ofDrugsshould be at minimal level todiscouragethegrowthofmicroorganisms during storage. Extractive values establish the amount of the active constituents. The extractions of any crude drug with a particular solvent yield a solution containing altered phytoconstituent. The compositions of these phytochemicals depend upon the nature of the plant and the solvent used. Results of physico-chemical analysis of *Adhatodavasica*, *Tinosporacordifolia*, *Glycyrrhizaglabra*, *Boerhaviadiffusa*, *and EcliptaAlba*has been presented in Table-3.

Table – 3 physicochemical parameters of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and EcliptaAlba* powder

S.No.		Observation				
Physicochemical Parameter		***Adhatodavasica***	***Tinosporacordifolia***	***Glycyrrhizaglabra***	***Boerhaviadiffusa***	***Eclipta alba***
1	Total ash value	12.5 %	6.74 %	6.05 %	9.24 %	17.43 %
2	Acid-insoluble ash value	0.44 %	1.64 %	0.70 %	3.19 %	8.57 %
3	Water soluble ash value	1.08 %	1.54 %	0.84 %	2.11 %	1.47 %
4	LOD	4.21 %	4.63 %	4.44 %	3.29 %	4.12 %
5	pH 1% Solution	6.54	6.47	6.55	5.48	6.10

6	Foreign Matter	0.62 %	0.71 %	0.24 %	0.91 %	0.78 %
7	Alcohol soluble extractive value	13.27 %	4.18 %	17.37 %	2.76 %	14.43 %
8	Water soluble extractive value	28.10 %	13.40 %	25.19 %	10.34 %	25.02 %

Antimicrobial Activity

Results obtained in the present study revealed that tested extracts possess potential antibacterial activity against *E. coli*, and *S. aureus*, when tested by disc diffusion method the chloroform, Ethanol, Aqueous extracts showed most promising results. The maximum ZOI has been observed with chloroform extract 24 mm has been observed against *S. aureus*, and 20 mm *E. coli* and least with Hexane extract. Ethyl Acetate extract of *Boerhaviadiffusa*exhibit highest activity against *S. aureus* of 20 mm and *Adhatodavasica*extract haveleast against *E.coli* 13 mm. Ethanol extract of *Adhatodavasica* showed maximum activity against *E. coli* 21 mm and least ethanol extract of *Glycyrrhizaglabra* 14 mm against *E. coli*. Hexane extract of *Adhatodavasica* and *Ecliptaalba* showed equal and highest activity against *E. coli and S. aureus* as 10 mm and least activity of hexane extract of *Boerhaviadiffusa* as for *E.coli*9 mm. Aqueous extract also have a good activity among all the extracts tested. The data pertaining to the antimicrobial potential of the plant extracts are presented in Table-4.

It is clear from the Table-4 that antibacterial activities of different extracts are showing promising results. *The growth inhibition zone measured ranged from 9 mm to 24 mm.* Trend of the activity of different extracts against *E.coli* and *S. aureus* is Chloroform> Ethanol > Aqueous > Ethyl acetate >Hexane. Maximum ZOI has been observed for Chloroform extract *i.e.* 24 mm and least ZOI for Hexane i.e. 9 mm.

Table – 4 zone of inhibition (in mm) of different extracts of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and Ecliptaalba* against E.coli (ATCC-8739)&*S. aureus (ATCC-6538)*

S.No.		Observation									
Plants ⇒		*Adhatodavasica*		*Tinosporacordifolia*		*Glycyrrhizaglabra*		*Boerhaviadiffusa*		*Eclipta alba*	
Organisms ⇒		***E. coli*** (ATCC-8739) (Gram - ev)	***S. aureus (ATCC-6538)*** **(Gram + ev)**	***E. coli*** **(ATCC-8739) (Gram - ev)**	***S. aureus (ATCC-6538)*** **(Gram + ev)**	***E. coli*** **(ATCC-8739) (Gram - ev)**	***S. aureus (ATCC-6538)*** **(Gram + ev)**	***E. coli*** **(ATCC-8739) (Gram - ev)**	***S. aureus (ATCC-6538)*** **(Gram + ev)**	***E. coli*** **(ATCC-8739) (Gram - ev)**	***S. aureus (ATCC-6538)*** **(Gram + ev)**
Extracts ⇓											
1	Aqueous + ve Control -ve Control	15 20 0	18 22 0	14 20 0	17 22 0	13 20 0	15 22 0	14 20 0	16 22 0	13 20 0	16 22 0
2	Hexane + ve Control -ve Control	10 20 0	12 22 0	11 20 0	13 22 0	12 20 0	14 22 0	09 20 0	13 22 0	10 20 0	12 22 0
3	Chloroform + ve Control -ve Control	20 20 0	24 22 0	17 20 0	21 22 0	19 20 0	23 22 0	18 20 0	22 22 0	15 20 0	19 22 0
4	Ethanol + ve Control -ve Control	17 20 0	21 22 0	16 20 0	18 22 0	14 20 0	17 22 0	16 20 0	18 22 0	15 20 0	19 22 0
5	Ethyl acetate + ve Control -ve Control	13 20 0	15 22 0	14 20 0	16 22 0	15 20 0	17 22 0	16 20 0	20 22 0	15 20 0	17 22 0

Discussion

Plants are rich in secondary metabolites like tannins, terpenoids, alkaloids and flavonoids and these secondary metabolites are responsible for antibacterial properties. The use of plants and its preparations to treat diseases is an ancient practice in world especially in developing countries like India where there is dependence on traditional medicine. Interest in plants with antibacterial properties has revitalized as a result of current problems associated with the use of antibiotics. The present studies aimed at the investigation of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and Ecliptaalba*ethnomedicinal plants *in vitro* antibacterial activity against Gram positive and Gram negative bacteria. The results presented here point out that these plants have a good choice for the development of new "leads". Hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and Eclipta alba* extracts showed significant zone of inhibition against "Gram-positive" bacteria, *Staphylococcus aureus A*TCC 6538 and Gram-negative bacteria *and Escherichia coli* ATCC 8739. This work shows that maximum ZOI has been observed in Chloroform extracts and least in hexane extracts. This means active components showing better antibacterial property are more lipophilic as compared to non polar solvent. Phytochemicals such as alkaloid are generally reported in Chloroform extract.[44] Comparing results found in this study with those of the literature, we notice in a previous work on antimicrobial activity of some medicinal plants from Tunisia, that methanolic extracts of *C. monspeliensis*leaves have shown an interesting activity against *P. aeruginosa, S. aureus, E. faecalis*with inhibition zones diameters of 18.0, 20.0 and 15.0 mm, respectively.[45] Whereas, water-methanol extracts of fruit peels of pomegranate (*P. granatum*) have demonstrated a moderate activity when they were tested on *S. aureus, P. aeruginosa and K. pneumonia*e (13.0, 18.0 and 16.0 mm, respectively)[46]. This activity of pomegranate peels could be attributed to tannins, for which antimicrobial activity has been demonstrated.[47] The studies commenced here also suggest that presences of good antibacterial potency of the extracts are due to active compounds in these extracts. The results indicate that the tested crude extracts are potential source to be explored to identify new compounds. As these plants are used in Ayurvedic formulations the results also revealed the scientific basis of the traditional usage of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa, and Eclipta Alba* and therefore received attention. This is supporting document to prove that these plants have therapeutic uses since ancient times. The use and exploration for drugs and dietary supplements derived from these plants have accelerated recently but much work has to be done.

Conclusion

Pharmacognostic evaluation plays an important role in quality control of the crude drug. The different characters observed in the *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra, Boerhaviadiffusa , and Eclipta alba* serve as base for the identification of right sample of the plant as drug and other studies. Five solvent extracts have been selected out of which Chloroform extracts, ethanol and water extracts have shown more promising results as compared to hexane and Ethyl acetate extracts. It can be concluded from this study that chloroform, ethanol and water are more suitable for further studies. Antibacterial leads seem to be more lipophilic in nature. The ZOI in chloroform extract is found to be even more as compared with standard drug Steptomycin against *S.aureus A*TCC 8739. The present study justified the claimed uses of *Adhatodavasica, Tinosporacordifolia, Glycyrrhizaglabra,*

Boerhaviadiffusa, and Ecliptaalba in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the prospective efficacy of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation for the potential discovery of new natural bioactive compounds.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

References

1. PMCID:PMC3672862
2. Rates, SMK. Plants as source of drugs. *Toxicon*., 2001, 39(5):603-13. doi:10.1016/ S0041-0101(00)00154-9.
3. Sharma, A.;Shanker, C.;Tyagi, L.K.; Singh, M. and Rao, C.V. HerbalMedicine for MarketPotential in India: An Overview. *Acad. J. Plant Sci*., 2008,1(2):26-36. doi:10.1177/1534582306289130.
4. Vaidya, A.D.B. and Devasagayam, T.P.A. CurrentStatus of HerbalDrugs in India: An Overview. *J. Clin. Biochem. Nutr*., 2007,41(1):1–11. doi:10.3164/jcbn.2007001.
5. Newman, D.J.;Cragg, G.M.; Newman, D.J. andCragg, G.M. Natural Products as sources of New Drugs over the Last 25 Years. 2007,70:477. doi:10.1021/np068054.
6. http://health.economictimes.indiatimes.com/news/pharma/indias-share-in-global-herbal-medicinal-market-is-0-5-pcgovt/55505280 (Accessed on 24 November, 2016)
7. Fabricant, D.S. and Farnsworth, N.R. The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect*., 2001, 109 (SUPPL. 1):69-75.doi:10.1289/ehp.01109s169.
8. Shahriar, M. Phytochemicalscreenings and Thrombolyticactivity of the leafextracts of A*dhatoda*vasica. The experiment*International Journal of science and technology*, 2013,7(4):438-441.
9. Arabind, K.V.K;Garg, R.K.; Singh, L. and Chauhan, S.S. PharmacognosticStudy and Establishment of QualityParameters of Leaves of *Adhatodavasica*. *Linn. Journal of Medicinal Plants Studies*, 2013,1(3):35-40.
10. Jain, M.P.V. ; N.G. and Atal, C.K.Recentadvances in vasakaalkaloids- a reviewIndiandrugs, 1984,21:313.
11. Chakraborty, A. andBrantner, A.H. Study of alkaloids from *Adhatodavasica*Nees on their anti-inflammatory activity. PhytotherRes., 2001,15:532.
12. Mulla, A.W. and More, D.S. Evaluation of anti-inflammatory and analgesic activities of ethanolicextract of roots *Adhatodavasica*Lin. *Int. J. Pharm. Tech.*Res., 2010,2(2):1364-1368.
13. Sheeba, B.J. and Mohan, T.S.Antimicrobialactivity of *Adhatodavasica*againstclinicalpathogens. *Asian J. Plant.Sci.Res.*, 2012, 2(2):83-88.
14. Bhatt, M.;Gahlot, M.;Juyal, V. and Singh, A. Phytochemical investigation and antidiabeticactivity of adhatodazeylanica. *Asian J. Pharm. Clin.Res*., 2011,4(2):27-30.
15. Vinothapooshan, G. and Sundar, K.Woundhealingeffect of variousextracts of Adhatodavasica. *IJPBS*, 2010,1(4):530- 536.
16. Ganguli, M.D. andParamesh, R. Clinicalevaluation of Evecaresyrup in the treatment of infertility in women:

An open study. *IJCP*, 2010, 20(11):767-771.

17. Vinothapooshan, G. and Sundar, K. Anti-ulceractivity of *Adhatodavasica*leavesagainstgastriculcer in rats. *JGPT*, 2011,3(2):7-13.

18. Kavitha, G. ;Thenmozhi, S. andRajan, S. Screening of Antibacterial and phytochemicalactivity of *Adhatodavasica*L againstclinicallyisolatedrespiratorypathogens. *IJPRBS*, 2012, 1(4):203-214.

19. Mahajan, N.;Dhar,K.l.; Suri, O.P.;Nepali, K.Kamra and N,Garg A.*et al*. Synthesis of some N-heterocyclic analogues of vasicine. *IJPSR*, 2010,1(2):78-86.

20. Patwardhan, B. and Gautam, M.Botanicalimmunodrugs: scopeandopportunities. *DrugDiscov. Today*,2005,10:495-502.

21. Patil. M ;Patki. P ;Kamath, H.V. andPatwardhan, B.Antistressactivity of *Tinosporacordifolia*(wild) Miers. *IndianDrugs*, 1997,34:211-215.

22. Anonymous, *IndianPharmacopoeia*, *Guduchi*(Published byIndian Pharmacopoeia Commission, Govt. Of India), 2007,2037-2034.

23. Anonymous,TheAyurvedicPharmacopoeiaofIndia(Published by Department Of AYUSH, Ministry of HealthAndFamilyWelfare,Govt. ofIndia,NewDelhi),Part-1,Vol.1,1999,53-55.

24. Singh, S.S.; Pandey, S.C.; Srivastava, S. Gupta, V.S.; Patro, B. and Ghosh, A.C. Chemistryandmedicinalpropertiesof*Tinosporacordifolia*(Guduchi). *Indian J. Pharm.*,2003, 35: 83-91.

25. Chintalwar, G.; Jain, A.; Sipahimalani, A.; Banerji, A.; Sumariwalla, P.; Ramakrishnan, R. and Sainis, K.Aimmunologicallyactivearabinogalactanfrom*Tinosporacordifolia*. *Phytochemistry*,1999, 52:108 9-1093.

26. Bisset,N.andNwaiwu,J.Quaternary Alkaloids of *Tinospora*species, *Planta. Med.*, 1983,48:275-279.

27. Jana, U.; Chattopadhyay, R.N. and Shaw, B.P.Preliminarystudieson anti-inflammatoryactivity of *Zingiber officinale* Rosc.,*Vitex negundo* Linn. and *Tinosporacordifolia*(Willd) Miersinalbinorats. *IndianJ. Pharm.*,1999,31:232-33.

28. Bishayi, B.; Roychowdhury, S.; Ghosh, S. and Sengupm, A.M. Hepatoprotectiveandimmunomodulatorypropertiesof*Tinosporacordifolia*in CCl_4 intoxicatedmaturealbino rats. *J.Toxicol. Sci.*,2002, 27:139-46.

29. StanelyMeinzen Prince,P.and Menon, V.P.Antioxidanteffectof*Tinosporacordifolia, Phytother. Res.*,2001, 15:213-5.

30. Chopra, R.N. ; Nayar, S.L. and Chopra, I.C. *GlossaryofIndianMedicinal plants*, (PublicationandInformationDiroctrate, NewDelhi),1956.

31. Singh, S.S.;Pandey, S.C.; Srivastava, S.; Gupta, V.S.;Patro, B. and Ghosh, A.C.Chemistry and medicinalproperties of *Tinosporacordifolia*(Guduchi),*Indian J. Pharmacol.*,2003, 35:85-91.

32. Agnivesa, elaborated by Charak and DradhbalawithAyurveddeepikacommentary by ShriChakrapanidutta, Edited by: VaidyaYadavjitrikamjiacharya, Prolouged by: Prof.R.H.Singh, CharakaSamhitaPublished by: ChaukhambhaSubharatiPrakashan, 2011, 31.

33. Robert Bently and Henry Trimen. Medicinal plants, FiftheditionPublished byAsiaticpublishing house, Delhi, 2,2007.

34. Najam, A. ; Singh, A. K. andVerma, H. N. Ancient and modern medicinalpotential of Boerhaavia diffusa and Clerodendrumaculeatum, *Research in Environment and Life Sciences*, 2008, *1*(1).

35. Chopra, R. N.; Ghosh, S.; Dey, P. and Ghosh, B.N. Pharmacology and therapeutics of Boerhaavia*diffusa* (Punarnava). *IndianMedical Gazette*, 1923,68:203– 208.

36. Chopra, R.N.; Nayar, S.L.;and Chopra, I.C. Glossary of IndianMedicinal Plants. *Council of Scientific and IndustrialResearch (CSIR), New Delhi, India*, 1956,34:39.

37. Bajpay, A. *EcologicalStudies of Boerhaaviaverticillata*PoirwithSpecial Reference to Phytochemical and Therapeutic Importance. Ph.D. Thesis; BanarasHinduUniversity, Varanasi, India, 1993.

38. Debjit, B.; Kumar1 K.P.; Sampath, Shweta, S.; Shravan, P.; Amit, S. andDutta, D. TraditionalIndianherbsPunarnawa and itsmedicinal importance,*Journal of pharmacognosy and phytochemistry*, 2012,*1*(1),55.

39. The Wealth of India, A dictionary of Indianrawmaterial and Industrialproducts, Vol.1, NISCAIR, CSIR, New Delhi, 2003, 47.

40. Sharma, P.C. ;Yelne, M.B. and Dennis, T.J.Database on Medicinal plants used in Ayurveda, Central council for research in Ayurveda and Siddha, Vol.2, Dept of ISM and H, 2001,112-115.

41. WHO guidelines. Quality control methods for medicinal plant materials. WHO Geneva, 1998:8-78.

42. Narayana, K. ; Kumar, S. ;Saraswathy, A. andAmerjothy, S. AntimicrobialPotential of Helicanthuselastica (Desr.) Danser A lessexploredIndianmistletoeGrowing on Mango Trees. *Journal of Traditional and ComplementaryMedicine*, 2014, 4(4):258–62.https://doi.org/10.4103/225-4110.126183.

43. Bhalodia, N.R. ; Nariya, P.B. and Shukla, V.J. Antibacterial and antifungalactivityfromflowerextracts of Cassia fistula L.: An ethnomedicinal plant. *Int. J. Pharm. Tech. Res*., 2011, 3(1):160-68.Doi:10.1016/j.jep.2007.04.008.

44. Khandelwal, K.R. Practical Pharma-cognosy Techniques and Experiments. Niraliprakashan:pune. ed. 9,2003.

45. Bensassi, A. ;Harzallah-Skhiri, F. andAouni, M. Investigation of somemedicinal plants fromTunisia for antimicrobialactivities. *Pharm. Biol*., 2007,45(5):421-8.

46. Al-Zoreky, N.S. Antimicrobialactivity of pomegranate (*Punicagranatum*L.) fruit peels. *Int. J. Food Microbiol*., 2009,134(3):244-8.

47. Cowan, M.M. Plant Products as Antimicrobial Agents. *Clin. Microbiol. Rev*., 1999,12(4):564-82.